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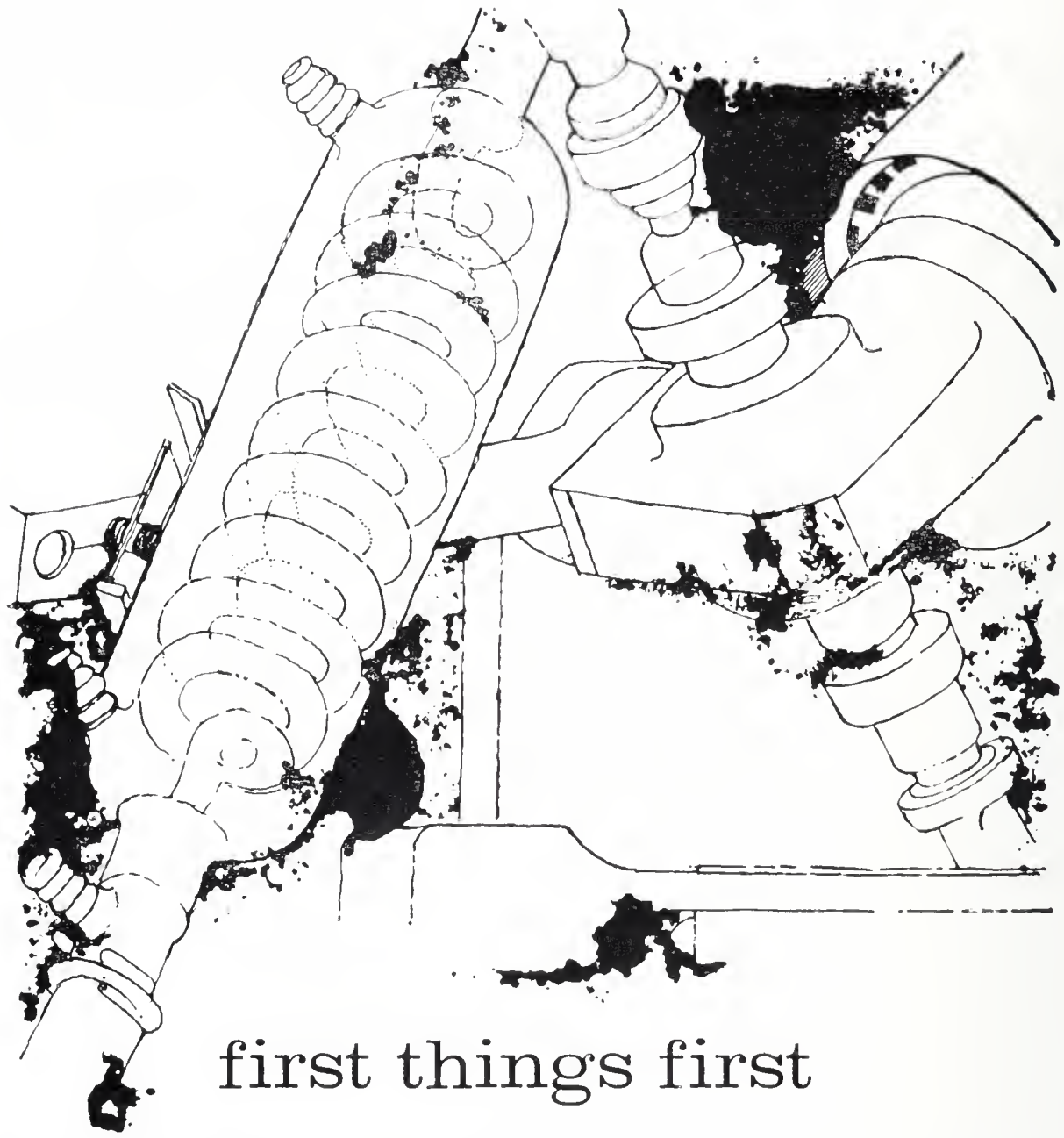

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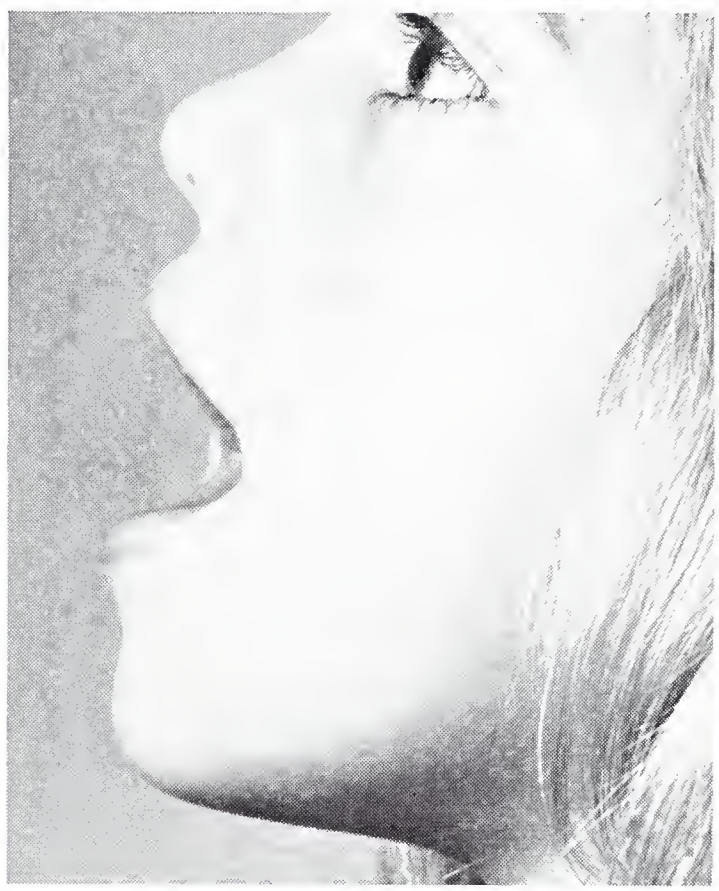

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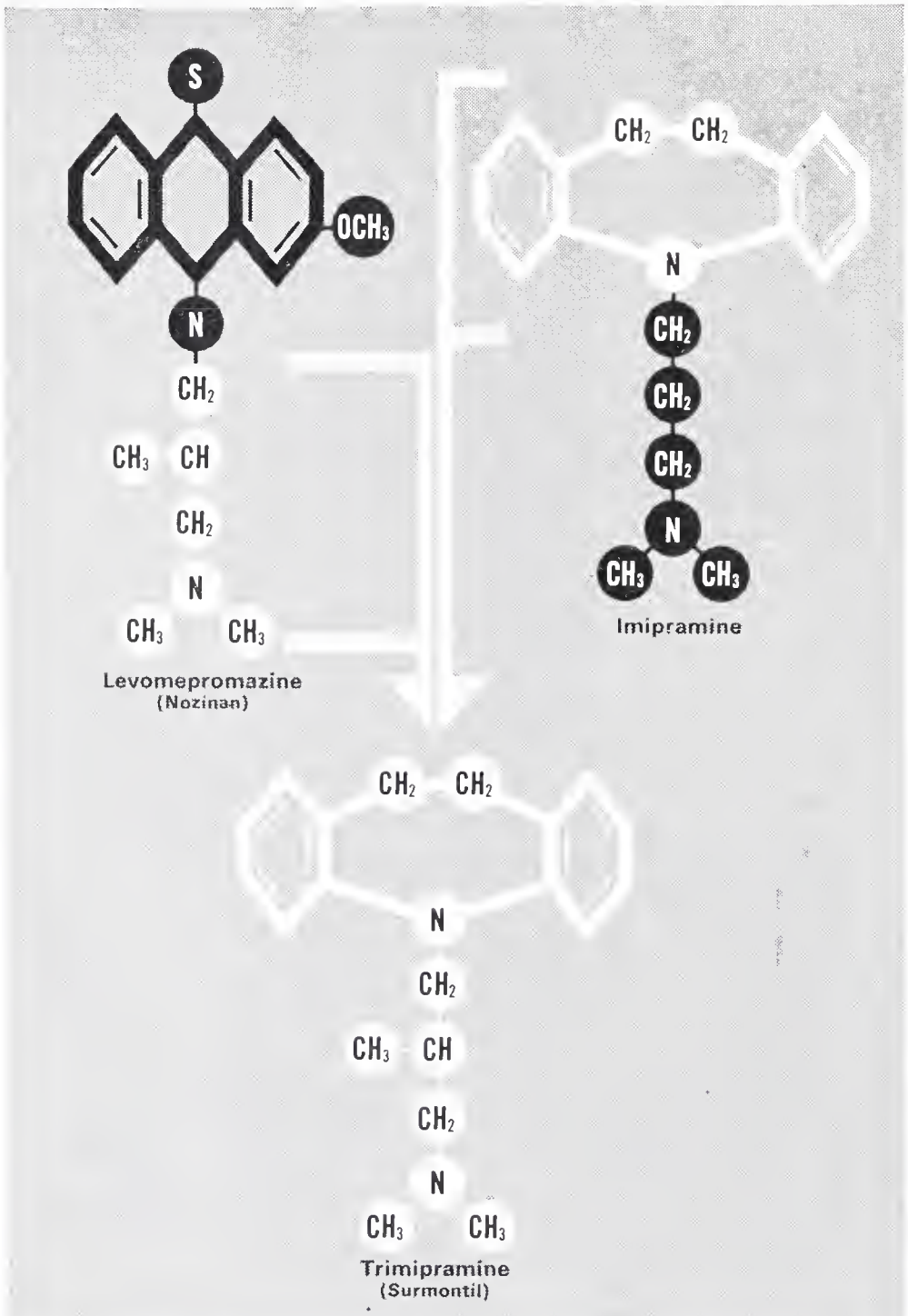

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Editorials

There are few areas of medical investigation which offer as much hope for contributing to man's knowledge and control of disease, as does the study of the immune mechanism. The immunological system is essential to health by virtue of its role in the destruction of foreign proteins. However, this same system seems able to contribute to disease in the form of hypersensitivity and autoimmune reactions. In addition, it is the integrity of the immune mechanism which makes organ transplantation such a difficult problem. This widespread involvement of the immunological system in the etiology, pathogenesis and treatment of disease, renders the need for adequate research in this field an urgent matter indeed. When the details of the immune mechanism are understood, when *specific* immune reactions can be augmented or suppressed, a great step toward the ultimate eradication of disease will have been made.

This journal, devoted to the topic of immunity and hypersensitivity, cannot hope to deal with more than a minute portion of the vast amount of work done in this field. We have chosen to concentrate on certain areas of investigation which are, in a number of ways, representative of the diversified aspects of immunology.

Much of the immunologists' work has dealt with the elucidation of the properties of the immunoglobulins in health and disease. This study of the immune mechanism on a molecular basis offers the only hope for the eventual control of specific immune reactions, and the eventual synthesis of specific antibodies in the laboratory. For these reasons, the first two papers in this issue review the present knowledge of the immunoglobulins.

The third paper is a product of the fact that in recent years the concept of autoimmunity as a cause of disease has burst into the limelight of clinical medicine. Although it is very tempting to accept an etiological role for the autoimmune reaction in a number of diseases, it is important to realize that the vast majority of the evidence is circumstantial and inconclusive. The three-part paper on myasthenia gravis and the thymus emphasizes this point by reviewing the evidence which has led many authorities to suggest that myasthenia gravis is an autoimmune disease.

The suggestion that abnormal immune mechanisms might also contribute to tumorigenesis is a fascinating and reasonable hypothesis. Accordingly, many investigations have been carried out in an effort to validate an immunological theory of cancer. The paper on tumour antigens provides a comprehensive survey of the theories that have evolved from the results obtained. The plausibility of the theories advanced makes it difficult to ignore the immune response when considering the factors which might be involved in causing cancer.

The final paper deals with the investigation of a case of autoimmune haemolytic anemia, a condition with which every clinician is familiar, but one which seldom is a complication of infectious mononucleosis. The final paper in this issue is an account of such a rare occurrence, in which the responsible antibody was identified. This report, based on original work done by a medical student during his summer vacation, emphasizes the value of the proper use of available laboratory methods in research problems.

It is the hope of those who have contributed to this issue that they have provided some insight into the rapidly growing study of immunology. The clinicians of the future or, for that matter, of the present, would be wise to keep abreast of the exciting developments in this field.

K.H.S.

The three-part article, Myasthenia Gravis and the Thymus: Fact or Fancy, is excerpted from a paper presented by the authors to the Alpha Omega Honour Medical Society. To our knowledge it has not been the rule for such papers to be published, but hopefully the printing of this article will set a precedent. Seven comprehensive, well researched topics are presented each year to the AOA Society; it sems a pity that a wider audience should not benefit from them.

B.E.K.

The role of the polymorphonuclear leukocyte in allergic inflammation has recently been the subject of intensive investigation. In the Arthus reaction, a classic example of allergic inflammation, antigen-antibody precipitates accumulate in the lumen, walls, and perivascular tissues of venules, followed by an accumlation of leukocytes.[1] The chemotaxis of leukocytes by antigen-antibody precipitates is probably due to the action of a heat-labile serum factor (presumably complement) which has been demonstrated *in vitro*[2] and may be the same as the permeability factory (PF/P) of Davies and Lowe.[3] The chemotaxis may be inhibited if one uses a "de - complemented" animal.[4]

Once leukocytes accumulate, they immediately begin to phagocytose the antigen-antibody precipitates (immune complexes).[5] With the electron microscope, Dr. H. Z. Movat has shown that the granules of the leukocyte enter the phagocytic vacuole containing the immune complexes and become lysed,[1] and eventually the cell becomes completely degranulated.[6] Thus, there is an intracellular reaction between antigen-antibody precipitates and leukocytic granules. Macroscopically, an inflammatory reaction (edema and hemorrhage) occurs. However, if an animal is made leukopenic with nitrogen mustard, these severe inflammatory changes are inhibited even though immune complexes can be demonstrated by the immuno-fluorescent technique.[5] It would seem, therefore, that the leukocytes, and particularly the leukocytic granules, are major factors in understanding the pathogenesis of allergic inflammation.

The leukocytic granules are rich in hydrolytic enzymes (acid phosphatase, alkaline phosphatase, nucleotidase, ribonuclease, lysozyme, cathepsin) and are analogous to lysosomes.[7] When the leukocytic granules enter the phagocytic vacuole containing the immune precipitates and become lysed, presumably proteolytic enzymes are released which break down the precipitates. From this reaction either a degradation product of the immune complexes (probably a polypeptide) or the granulocytic enzymes themselves are released which induce an increased vascular permeability and the resultant inflammation.[8]

While the granular factor which increases the vascular permeability has not yet been identified, the hypothesis has been validated by the demonstration that leukocytic granules enhance the Arthus reaction when injected intradermally and partly restore the reaction in leukopenic rabbits.[9] Isolated leukocytic granules, both intact and lysed, incubated with immune complexes result in an increase in vascular permeability.[8] Work is being done to find an inhibitor to the granule permeability factor. At the present time experiments have shown that Trasylol (Bayer 3380), a kallikrein inhibitor, will inhibit vascular permeability when incubated with immune complexes and rabbit peritoneal leukocytes.[10]

The importance of leukocytic granules in allergic inflammation is only now being understood. Moreover, this mechanism of inflammatory reaction will probably be applicable, not only to allergic, but also to other forms of inflammation.

J.S.B.

REFERENCES

1. Uriuhara, T., Movat, H. Z. Lab. Investig. 13: 1057 - 1079, 1964.
2. Boyden, S. J. Exp. Med. 115: 453, 1962.
3. Davies, G. E., Lowe, J. S. Int. Arch. Allergy 20: 235 - 250, 1962.
4. Movat, H. Z., Uriuhara, T., Burke, J. S.: Unpublished Observations.
5. Cochrane, C. G., Weigle, W. O., Dixon, F. J.: J. Exp. Med. 110: 481, 1959.
6. Daems, W. T., Oort, J.: Exp. Cell Res. 28: 11, 1962.
7. Cohn, Z. A., Hirsch, J. G.: J. Exp. Med. 112: 983 - 1104, 1960.
8. Movat, H. Z., Uriuhara T., Macmorine, D. L., Burke, J. S.: Life Sci. 3: 1025 - 1032, 1964.
9. Thomas, L.: Proc. Soc. Exp. Biol. Med. 115: 235 - 240, 1964.
10. Burke, J. S., Uriuhara, T., Macmorine, D. R. L., Movat, H. Z.: Life Sci. 3: 1505-1512, 1964.

The Immunoglobulins: Structure, Production and Function

M. Schwartz 6T

The immunoglobulins are a system of related proteins which are the products of the plasma cells.[29] They have been found to be a rather heterogeneous group, both physically and chemically, and include proteins of β and γ electrophoretic mobility. The term "γ-globulin" originally defined those proteins which migrate in one broad region of the electrophoretic spectrum, but it has since acquired an immunological connotation which is not limited to this region. The immunoglobulins can be divided into four classes:

γ_{2S}-globulin

γ_{1A}-globulin

γ_{1M}-globulin

γ_{u}-globulin (urinary, low molecular weight).

Some of the characteristics of the four classes and some of the many alternate names for them are listed in Table I.

Much of our knowledge of the immunoglobulins has been obtained from the study of the serum and urinary proteins of patients with multiple myeloma and Waldenström's macroglobulinemia. The hugely elevated levels of protein in the serum and urine of these people make possible the collection of large samples of protein from single patients. It was originally thought that these "paraproteins" were abnormal in structure, but comparisons with samples from normal individuals suggest that they are not.[27] It seems, too, that the gross structure of rat[29] and rabbit[24] globulins, which have been used for much of the investigation of antibody specificity, is similar to that of human globulins.

TABLE I: SUMMARY OF THE IMMUNOGLOBULINS AND SOME EXPERIMENTAL FRAGMENTS

(After various sources)

	NAME ADOPTED BY THIS REVIEW	SYNONYMS	SEDIMENTATION CONSTANT IN SVEDBERG UNITS	APPROXIMATE MOLECULAR WEIGHT
	γ_{2S}-globulin	6.5S, 6.6S, 7S γ-globulin γ_2-globulin	6.6	150,000
	γ_{1A}-globulin	β_{2A}-globulin	7–13 (mostly 7)	150,000–500,000
	γ_{1M}-globulin	19S γ-globulin β_{2M}-globulin macroglobulin	19 (variable)	750,000–850,000
	γ_{u}-globulin (urinary)	microglobulins Bence Jones proteins (BJP)	monomer 3.7 dimer 5.3	20,000 41,000
Papain Fragments	S (slow) fragment [I plus I′]			$2 \times 45{,}000$
Papain Fragments	F (fast) fragment	fragment III		55,000
Polypeptide Chains	L (light) Chain	B chain	3.7	20,000
Polypeptide Chains	H (heavy) Chain	A chain		53,000

Most of the work on the immunoglobulins has been on γ_{ss}-globulin and γ_{u}-globulin. Analysis of the structure and function of these two classes is, therefore, most complete.

ENZYMATIC HYDROLYSIS

Under standard conditions, proteolytic enzymes, such as papain and pepsin, cleave γ_{ss} molecules into constant, reproducible fragments which can be separated by ion-exchange column chromatography. Early experiments with papain[1] produced fragments with different electrophoretic mobilities. It was found that fragments I or I′ (which proved to be identical) contained the active antibody combining site.[1] Figure I shows that these two fragments comprise the S (slow) fragment. One review[23] suggests that fragment III, the F (fast) fragment, may be responsible for complement fixation.

Subsequent experiments, using antisera specific to various enzymatically-produced pieces and polypeptide chains, have charted the common antigenic characteristics of the various subunits of the γ_{ss}-globulin molecule and have established the hypothetical model shown in figure I.[9 20 23]

Tryptic digestion, which breaks the molecule into many small peptide chains, has been used with two-dimension, high voltage, paper electrophoresis to elucidate amino acid sequence.[11] This is called "fingerprinting" because each peptide map so produced is peculiar to the protein from which it was obtained.

POLYPEPTIDE CHAINS

Reduction and alkylation with mercapto-ethanol in the presence of 6M urea has been used to degrade the γ_{ss}-globulin into two types of polypeptide chain that appear to be the structural units of the molecule. Because of their large difference in size, the two species, H (heavy) and L (light) chains, can be separated by column chromatography using Sephadex which separates particles on the basis of size alone. When H and L chains are subjected to electrophoresis in a starch-urea gel, it is found that the L chain migrates more quickly toward the cathode than does the H chain.[5] This difference is used as a criterion of purity in testing the fractions obtained by column chromatography. Two other methods which are also used to identify and check the purity of samples are ultracentrifugation and double diffusion precipitin reactions in agar (Ouchterlony plates). The latter method employs specific antisera and establishes the antigenic identity and purity of the sample.

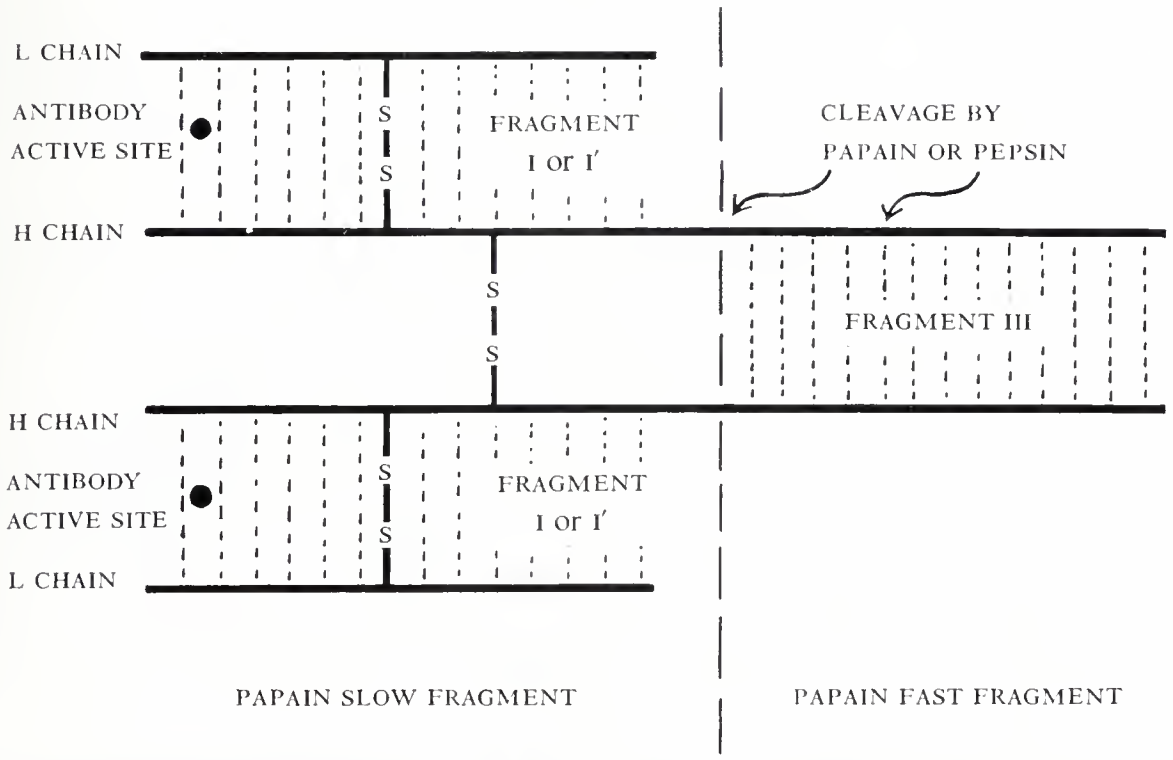
H CHAIN

The H chains of the three classes which have them (γ_{ss}, γ_{1A} and γ_{1M}) contain antigenic determinants which are specific for their class of immunoglobulin.[17] (See Figure II)

The H chain of γ_{ss}-globulin has a molecular weight of about 53,000. Attempts to resolve the H chains of this class into distinct electrophoretic components have

FIGURE I: POSSIBLE ARRANGEMENTS OF POLYPEPTIDE CHAINS IN γ_{ss}-GLOBULIN

(After A. Nisonoff, G. J. Thorbecke[23])



Papain with a reducing agent liberates 3 fragments (2 x I or I′ and III). Under certain experimental conditions a single electrophoretically slow fragment with the central disulfide bond intact may be released.
The antibody active site has been shown to be associated with the slow fragment.
Reduction of the three disulfide bonds in the presence of urea degrades the molecule into two L (light) and two H (heavy) polypeptide chains.

been unsuccessful, but it has been shown that their mobilities are related to those of the original γ_{ss}-globulins from which they came.[27]

L CHAIN

The L chain has a molecular weight of about 20,000. Whereas the H chains of the immunoglobulins are class specific, the L chains of the four classes are antigenically similar.[17] (See Figure II)

The L chains of the immunoglobulins of a normal person migrate electrophoretically as a diffuse band. Those of a person with multiple myeloma are localized into one or two distinct peaks which differ from patient to patient.[4]

By using antisera specific for the L chains of individual patients it was found that L chains exist in two molecular forms, type I and type II. The members of each family have antigenic similarities. Antiserum to one member cross-reacts with other members of the family.[17] (Figure II). The fingerprints of the two families are dissimilar.[11]

Numerous experiments have shown that the L chains are identical with the γ_u-globulins of the same patient. They have the same antigenic determinants, the same rate of migration in urea gel electrophoresis, identical amino acid composition and spectrofluorometric behaviour.[11] L chains and γ_u-globulins share the same peculiar solubility characteristics. They precipitate out of solution at about 50°C, and redissolve approaching the boiling point. As the solution is cooled, they reprecipitate, then redissolve.

ANTIBODY ACTIVITY OF γ_{ss}-GLOBULIN

It seems that antibody specificity is a function of the L chain[3, 12] and that antibodies to different antigens differ significantly in their amino acid sequence, as shown by fingerprinting.[3 13] It seems, too, that the interaction of both the H and the L chain is necessary for antibody action.[14] As shown in Figure I there are two antibody active sites per γ_{ss}-globulin molecule, each with an H and an L chain participating in the site.

BONDING OF γ_{ss}-GLOBULINS

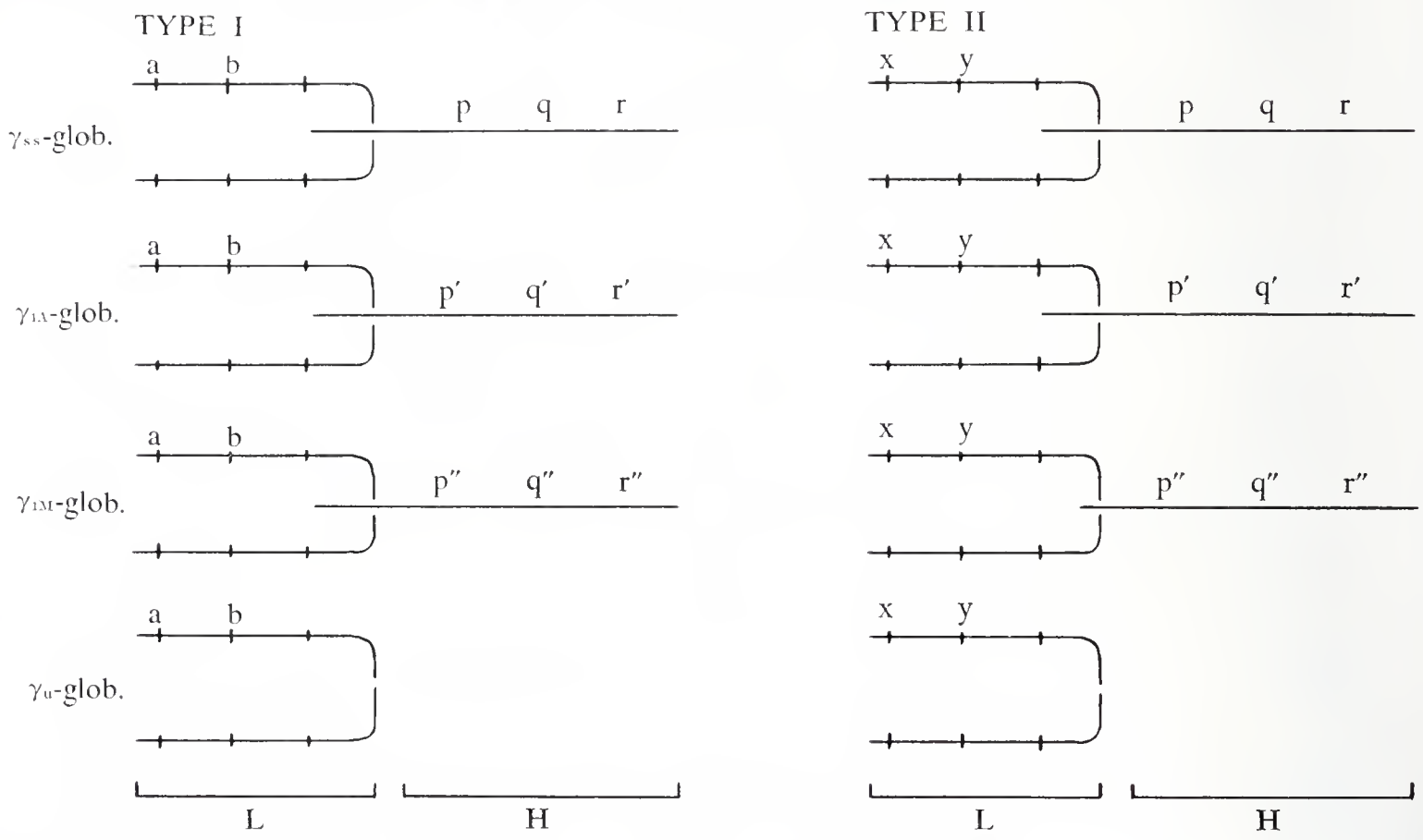
Various experimenters have shown that the γ_{ss}-molecule is composed of two H and two L chains schematically arranged as shown in Figure I.[9 23] The number and positioning of the disulfide bonds joining the chains is still uncertain.

Recent work[24] shows that the structural stability of the γ_{ss}-globulin molecule is due to both non-covalent forces and disulfide bonding, and establishes that cleavage and separation of the chains is reversible. It is

FIGURE II

(After J. L. Fahey[17])

Diagrammatic representation of relationships between the two families of molecules (I and II) in each of the four classes of the immunoglobulins. (γ_{ss}-globulins, γ_{1A}-globulins, γ_{1M}-globulins, γ_u-globulins).



The a, b symbols indicate type I antigenic determinants and x, y indicate type II determinants. The heavy chains contain antigenic determinants which are specific to each class of immunoglobulin.
The specific γ_{ss} (p, q, r) and γ_{1A} (p′, q′, r′) and γ_{1M} (p″, q″, r″) determinants are separately represented.

interesting to note that in this study hybrid molecules were produced consisting of rabbit L chains and human H chains, that retained many physical characteristics of γ_{ss}-globulin.

γ_u-GLOBULINS

Urine of normal subjects contains small amounts of γ_{ss}-globulin identical with the γ_{ss}-globulin of the patient's serum and a "low molecular weight" globulin (γ_u-globulin) which is identical to the L chain of the patient's serum immunoglobulins.[11 28] The level of γ_u-globulin is increased after exercise.[28] It is also increased in the diseases, multiple myeloma and Waldenström's macroglobulinemia, which are characterized by gross excess of serum globulin. It has been suggested[5 7] that in these diseases there is a production of L chains in excess of the H chains and that the γ_u-globulins are unincorporated L chains which overflow into the urine.

The γ_u-globulin is present in three forms in the urine,[26] a monomeric form (single L chain), an unstable dimeric form (2 L chains held together by noncovalent forces), and a stable dimeric form with an intact disulfide bond between the two L chains.

The L chains obtained as γ_u-globulins have been combined with the H chains of the same patient to form a product identical to his γ_{ss}-globulin.[26]

γ_{1M}-GLOBULINS

The γ_{1M}-globulins are more extensively discussed in other reviews.[6 21 23]

The γ_{1M}-globulins are present in small amounts in normal serum, and have their pathological counterparts in the paraproteins of Waldenström's macroglobulinemia. A "euglobulin" fraction, rich in γ_{1M}-globulin can be obtained from macroglobulinemic sera by virtue of the insolubility of γ_{1M}-globulin in cold, distilled water.

The γ_{1M}-globulins are variable in molecular weight and electrophoretic mobility (See Table I). Characteristically they have a sedimentation constant of 19S and a molecular weight approaching one million, but minor components range as high as 40S. Electrophoretic mobility ranges from the mid-gamma area to the beta region.

The L chains of this class cross-react with those of other classes,[17] but the H chains possess antigenic determinants distinct from other immunoglobulins.[17 20] In macroglobulinemia γ_u-globulins identical to the L chains of the serum γ_{1M}-globulins are found in the urine.[25]

In the course of immunization to a particular antigen, humans and laboratory animals initially produce γ_{1M} antibodies. Subsequently γ_{ss} antibodies to the same antigen are formed.[21 29] It seems that some antigens elicit the formation of γ_{1M}-antibodies only. Examples of these antigens are the somatic "O" antigens of typhoid bacilli. The rheumatoid factor found in the serum of some patients with rheumatoid arthritis is a γ_{1M}-globulin.

γ_{1A}-GLOBULINS

The γ_{1A}-globulins are likewise more extensively reviewed elsewhere.[6 21 23]

This class of immunoglobulin is the least known because contamination by other immunoglobulins in supposedly pure preparations has made investigation difficult. Like the γ_{1M}-globulins, they are heterogeneous in molecular weight and electrophoretic mobility. They vary from 7 to 13S and migrate in the fast gamma/slow beta region. Like the γ_{1M}-globulins, too, their L chains cross-react with those of the other classes, but their H chains are antigenically distinct.[17 20]

There is still some doubt as to whether or not the γ_{1A}-globulins have antibody activity.[21 23] One review[21] suggests that the following antibodies are γ_{1A}-globulins:

1. The skin sensitizing antibodies in allergic individuals (Prausnitz-Küstner reaction).
2. Anti-A isoagglutinins.
3. Horse antisera to diphtheria, tetanus toxins.

The γ_{1A}-globulins have their pathological counterparts in the sera of some patients with multiple myeloma.

SYNTHESIS OF THE IMMUNOGLOBULINS

It was indirectly shown,[10] then proven,[22 29] that the entire antibody molecule is synthesized in one cell.

The work being done today with single cell cultures is extremely fascinating. Animals are injected with antigen and given time to react. Then the lymph nodes draining the site of injection are removed and single cells are isolated and incubated.

Using the flagellar "H" antigen of different strains of Salmonella as the injected antigen, and the immobilization of active motile bacteria in the medium surrounding the incubating single cells as the criterion of antibody production, one laboratory[29 30] has demonstrated the production of strain-specific antibody by single cells. Moreover, a single cell produced antibody to one antigen only. It was found that the initial response of the single cell is the production of γ_{1M}-antibody. Then for a short time, the cell produces both γ_{1M}- and γ_{ss}-antibodies, then finally γ_{ss}-antibody only. Cells incubated from animals following a second injection of antigen demonstrate a more rapid switchover to γ_{ss}-antibody production than do those obtained following an initial injection. The L chains, or the combining sites, of γ_{1M}- and γ_{ss}-antibodies to the same antigen have not yet, however, been demonstrated to be chemically identical.

A second group of experimenters using virus inactivation as their criterion of single cell antibody production found that one cell was able to produce specific antibodies to several different antigens simultaneously.[22]

The first group's findings are compatible with a "clone theory" of antibody production wheras the findings of the second group are more suggestive of an "instructive theory". It would be interesting to see the response to antigenic stimuli of single undifferentiated cells isolated from non-immunized animals.

CONCLUSION

The immune response is the mechanism that delimits the biological self. Although we talk glibly about "the body's defences" and its "response to foreign invaders", nobody knows exactly what these are. Certainly antibodies are mobilized against large molecules that enter the body, and sometimes antibodies are turned inward against the host himself. At present, the immunoglobulins, their structure, production and function, are only poorly known. A better understanding of them is essential to a better understanding of the processes of health and disease.

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REFERENCES

1. Porter, R. R. Biochem. J. 73: 119, 1959.
2. Deutch, H. F., Stiehm, R. E., Morton, J. T. J. Biol. Chem. 236: 2216, 1961.
3. Edelman, G. M., Benacerraf, B., Ovary, Z., Poulik, M. D. Proc. Natl. Acad. Sci. U.S. 47: 1751, 1961.
4. Poulik, M. D., Edelman, G. M. Nature 191: 1274, 1961.
5. Edelman, G. M., Benacerraf, B. Proc. Natl. Acad. Sci. U.S. 48: 1035, 1962.
6. Fahey, J. L. Advan. Immunology 2: 42, 1962.
7. Edelman, G. M., Gally, J. A. J. Exptl. Med. 116: 207, 1962.
8. Olins, D. E., Edelman, G. M. J. Exptl. Med. 116: 635, 1962.
9. Fleischman, J. B., Porter, R. R., Press, E. M. Biochem J. 88: 220, 1963.
10. Fleischman, J. B. J. of Immun. 91: 163, 1963.
11. Schwartz, J. H., Edelman, G. M. J. Exptl. Med. 118 41, 1963.
12. Roholt, O. A., Radzimski, G., Pressman, D. Science 141: 726, 1963.
13. Koshland, M. E., Englcberger, F. M. Proc. Natl. Acad. Sci. U.S. 50: 61, 1963.
14. Olins, D. E., Edelman, G. M., Gally, J. A. Proc. Natl. Acad. Sei. U.S. 50: 753, 1963.
15. Franklin, E. C. J. Immun. 91: 73, 1963.
16. Fahey, J. L. J. Immun. 91: 438, 1963.
17. Fahey, J. L. J. Immun. 91: 448, 1963.
18. Fahey, J. L. and McLaughlin, C. J. J. Immun. 91: 484, 1963.
19. Vaerman, J. P., Heremans, J. F., Vaerman, C. J Immun. 91: 7, 1963.
20. Migita, S., Putnam, F. W. J. Exptl. Med. 117: 81 1963.
21. Mannik, M., Kunkel, H. G. Bulletin on Rheumatic Diseases, 13: 309, 1963.
22. Attardi, G., Cohn, M., Horibata, K., Lennox, E. S J. Immun. 92: 335, 1964.
23. Nisonoff, A., Thorbecke, G. J. Ann. Rev. Biochem 33: 355, 1964.
24. Olins, D. E., Edelman, G. M. J. Exptl. Med. 119: 789 1964.
25. Gross, D., Epstein, W. V. J. Clin. Invest., 43: 83 1964.
26. Gally, J. A., Edelman, G. M. J. Exptl. Med. 119: 817 1964.
27. Cohen, S., Porter, R. R. Biochem. J. 90: 278, 1964.
28. Freedman, M. H., Connell, G. E. Can. J. Biochem. 42: 1065, 1964.
29. Nossal, G. J. V., Szenberg, A., Ada, G. L., Austin C. M. J. Exptl. Med. 119: 485, 1964.
30. Nossal, G. J. V. Sci. Am., 211: 6, 106, 1964.

The Immunoglobulins in Health and Disease

S. Presant 6T6

Normal human serum contains a complex mixture of proteins which have been separated traditionally by precipitation methods;[1] more recently, methods based on physical properties, ultracentrifugation and electrophoresis have emerged. The gamma globulins are defined arbitrarily as that group of proteins which move more slowly when serum is submitted to electrophoresis at pH 8.6 in barbital buffer of 0.1 ionic strength. No sharp division exists between these globulins and the next large fraction, the beta globulins. In 1939, Tiselius and Kabat[2] demonstrated the association between precipitating antibody and gamma globulin, when they showed a striking rise of gamma globulin in the serum of strongly immunized animals while other fractions were unchanged. If the antibody was precipitated by adsorption onto the antigen, only the gamma globulin fraction showed a significant fall.

When non-lipid-bearing proteins are separated by ultracentrifugation, they fall into three major components, formerly designated the A, G, and M peaks.[3] The A component sediments more slowly, has a sedimentation coefficient of 4S, and is *albumin;* the G is intermediate in size and sedimentation rate (7S), and is almost entirely *gamma globulin*. The smallest of these peaks, the M peak, has a sedimentation coefficient of about 19S, sediments with great rapidity, and contains the heaviest proteins with molecular weights of about one million; their large size gave rise to the name, *macroglobulin*. The macroglobulins comprise about 6 percent of total serum protein,[4] and when they are separated from serum and examined by electrophoresis, about two-thirds migrate with a small group of proteins with a mobility between that of the beta and gamma globulins. This peak has been designated both the slow-moving beta and fast-moving gamma; that is, beta-2 or gamma-1. These macroglobulins have antibody activity. The other third of the 19S component migrates with the alpha-2 globulins, and is not currently believed to have antibody activity.

Another useful technique is immunoelectrophoresis.[5] Serum is first fractionated by electrophoresis in agar gel, and a trough is cut parallel to the line of electrophoresis. Anti-human serum is run into the trough, and the antigens and antibodies are allowed to diffuse toward each other until precipitation lines become apparent. Many lines form but the gamma globulin line starts with the slowest moving gamma-2 and runs through the gamma-1, beta and the alpha-2 fractions. This very broad overlap with other components is in sharp contrast to the results with the starch gel method, where the gamma globulin, though spread, is well separated from the alpha and beta globulins. These results, as well as others, suggest that antibody activity is not limited to the electrophoretic gamma globulins, but is distributed almost throughout the range of plasma globulins, and for these proteins, the new term, *immunoglobulin*, can be used. The immune globulins have molecular weights of 150,000 (7S globulins) or 1,000,000 (19S globulins). The 7S globulins can be separated by immunochemical techniques into two antigenically related but not identical classes, the beta-2 group with the bulk of molecules migrating in the beta-2 position, and the gamma-2 class with the bulk of molecules migrating more slowly than the former, and mainly accounting for the large gamma globulin hump seen on electrophoresis. Zone electrophoresis has been used to measure serum gamma globulin levels, but because some molecules of each of the three immunoglobulin groups migrate more rapidly than the electrophoretically defined gamma globulin region, the total amount of serum immunoglobulin has been underestimated. In addition, zone electrophoresis does not distinguish each gamma globulin group.

Using purified antibodies, Fahey and Lawrence[4] found the 7S gamma-2 globulins to be present in amounts of 12.63 ± 2.7 mg./ml., the beta-2 globulins to represent 3.94 ± 0.91 mg./ml., and the 19S beta-2 macroglobulins to represent 1.6 ± 0.55 mg./ml. normal serum. Thus, the immune globulins constitute 17.73 mg./ml. or 25 percent of serum proteins. These workers next separated normal serum by zone electrophoresis in polyvinyl particle blocks, which were divided into 35 fractions, each of which was examined for protein content and for each of the immunoglobulin components. The bulk of the 7S gamma-2 globulins were in the gamma electrophoretic region, but they extended in progressively diminishing amounts through the beta and into the alpha regions. The beta-2 globulins are most heavily concentrated in the slower half of the beta region, but extend into the adjacent alpha and gamma regions. The beta-2 macroglobulins are roughly intermediate in electrophoretic distribution, reaching their greatest concentration in the faster migrating gamma globulin region. The value of 25 percent for the fraction of total serum protein being gamma globulin (immune globulin) is larger than that obtained by conventional zone electrophoresis,[6] for reasons already noted.

CHEMICAL STRUCTURE

Details of the work in this area can be found elsewhere in this Journal.

METABOLISM[15]

(a) Normal

Localization of immunoglobulins, by fluorescent tracer technique, in plasma cells, and, in certain instances in lymphocytes, and occasionally in transitional cells resembling both types of cell, clearly implicated these cells in the origin of antibodies. This belief is further confirmed by the absence in patients with agammaglobulinemia, of plasma cells and occasionally lymphocytes, as well as one or more of the immune globulin subclasses.

Recent work has provided abundant evidence that 19S antibodies are formed early during the immune response, and that 7S antibodies are formed later, provided the antigenic stimulus is sufficiently strong. Even the neonate and fetal sheep can form 19S antibody, and these neonatal antibodies are similar in all respects to those in adults.[14]

The total antibody of the body (about 80 g. in healthy adults) is distributed equally between circulating plasma and interstitial fluids, with about 25 percent of circulating antibody passing across capillaries to interstitial fluids each day, and a similar amount returning to the blood through the main lymphatic ducts. However, the distribution of 19S globulin differs from 7S globulin, in that only a smal amount of the total pool of the former is present in the extravascular fluids.

The 19S macroglobulins have a much higher turnover rate than 7S globulins; the latter group, despite physicochemical and immunologic heterogeneity, appears metabolically homogeneous, and is catabolized at a uniform rate. In healthy adults, the half-life of isotopically labelled 7S globulin is about three weeks, and the absolute turnover rate is 1.5 to 2.5 grams per day.[16]

(B) Abnormal

In many diseases, hyperglobulinemia may develop.[17 18] Chronic malarial infection is associated with high gamma globulin levels which are significantly reduced by antimalarial prophylactic therapy.[19] In cirrhosis associated with hyperglobulinemia, the 7S gamma-2 and 7S beta-2 globulins and 19S macroglobulins are all elevated. The half-life of isotopically labelled gamma globulin is significantly shortened, and the rate of gamma globulin synthesis increased. Strangely, in protein malnutrition in children (kwashiorkor), though there is always hypoalbuminemia, there may be hyperglobulinemia; the gamma globulin production, though usually related to the state of nutrition, is markedly increased by infection. In myelomatosis, in spite of the hyperglobulinemia so characteristic of the disease, the concentration of normal gamma globulin is often diminished, and susceptibility to infection increased.[2] The hyperproteinemia results from accelerated synthesis, and rates of synthesis of myeloma proteins twenty times that of normal gamma globulin have been found. The Bence-Jones protein is either a by-product or precurso of myeloma protein synthesis, and not a degradatio product of myeloma proteins. It seems that, in mye loma, excess synthesis of abnormal gamma globulin i often associated with asynchronous production of poly peptide chains, constituting the molecular failure t adequately assemble these chains. As a result, Bence Jones protein enters the blood and is excreted in th urine. In a case of hypoglobulinemia,[21] the rate of de gradation of labelled gamma globulin administered t the patient was slower than in two normal controls suggesting that in this case there was conservation o antibody in relation to normals.

RHEUMATOID FACTORS AND INHERITANCE OF GAMMA GLOBULIN[24]

There is an unusual protein in the serum of man patients with rheumatoid arthritis and certain other di seases. It is a macroglobulin with the physical an chemical properties of the 19S class of antibodies. Al though it has been produced in rabbits by hyperimmun ization with typhoid bacilli,[22] the bulk of evidence in dicates that these are antibodies to antigen thought t be 7S gamma globulin itself, and that the rheumatoi factor might be an anti-antibody. The rheumatoi factor circulates in the blood as a 22S complex as sociated with 7S gamma globulin. These complexe have been disassociated with acid and urea, and th isolated rheumatoid factor has been reassociated wit 7S antibody when mixed with whole serum or Fractio 11 globulin. One mole of rheumatoid factor appear to be combined with six moles of gamma globulin.[2] Clinically, assay is carried out by agglutination of a variety of indicator carriers coated with human gamma globulin derived from Cohn Fraction 11 (F11). Ben tonite and latex particles are currently preferred fo the test.

The bentonite and latex flocculation tests for rheumatoid factor utilize pooled human gamma globulin a antigen. Recently, Grubb and Laurell[25] coated humar red cells, type D^+, with a single donor human gamma globulin in the form of an incomplete Rh antibody (incomplete anti-D). Next, a single rheumatoid serum wa added and agglutination was frequently observed. Inhibition of the agglutination reaction was then attempted by mixing a single rheumatoid serum with a single donor human whole serum (a potential source of antigen, namely 7S globulin). Certain sera did inhibit the agglutination of the indicator system (D^+ cells with incomplete anti-D) while others did not. As expected, the ability to inhibit the agglutination was found to reside in the 7S fraction of whole serum. Clearly, a single rheumatoid factor would react with the 7S gamma globulin present in some, but not all sera. In family studies it was shown that the inhibiting type of gamma globulin was inherited according to simple genetic laws.

The symbol Gm was adopted for gamma globulin, and the initially discussed factor found in only some of

the 7S gamma globulin was designated Gm(a). The gamma globulin which could inhibit the particular reaction was called Gm(a+), and that which could not Gm(a−). The agglutinator in rheumatoid sera (the rheumatoid factor) was called anti-Gm, in this case, anti-Gm(a+). Subsequently, it was shown that the gamma globulins used as cell coats, the incomplete anti-D's, are of the same Gm type as the inhibiting sera. A rheumatoid serum containing anti-Gm(a) will agglutinate a cell coated with a Gm(a+) incomplete anti-D, and will be inhibited by a Gm(a+) gamma globulin in appropriate dilutions. By knowing the specificity of any two of the components of the test, that of the third can be determined. If the anti-Gm and incomplete anti-D specificities are known, the unknown gamma globulin can be typed. If the cell coat and inhibiting specficity are known, the anti-Gm specificity can be determined. Using the same methods, by selecting various rheumatoid sera and cell coats, and by doing family studies, many other Gm types have been reported.

Genetic Control

The synthesis of the immunoglobulins in man is under the control of at least two separate and distinct genetic loci, the Gm already discussed and another designated Inv.[14] The former is found only in 7S gamma-2 molecules, the latter being expressed in all classes of immune globulins. The existence of two loci specific for 7S beta-2 and 19S beta-2 globulins seems certain. Each of these loci regulates the synthesis of a single type of polypeptide chain. The Inv locus, expresses itself on the A fragment (L chain) common to all immune globulins, while Gm activity is limited to 7S gamma-2 globulin, and consequently expresses itself on fragment B (H chain). It seems reasonable to suppose that unidentified loci direct the synthesis of H chains in the other proteins.

Several family studies have shown that the two major alleles at each of the known loci; namely, Gm (a) and Gm (b), and Inv (a) and Inv (b); express themselves as codominants, at least in Caucasians. The frequency of the various Gm and Inv phenotypes differs considerably in different ethnic groups. In addition, certain genes appear restricted to Caucasian populations, whereas others apparently are found only in non-Caucasians.[24]

NEONATAL IMMUNITY[3]

Cord serum is deficient in beta-2 globulins and beta-2 macroglobulins. The newborn is uniquely susceptible to infection by gram-negative enteric bacilli, most of which are not ordinarily pathogens in later life. This is, perhaps, at least partly because natural bacteriocidins against these bacilli are 19S macroglobulins, and are, therefore, absent from cord blood. These bacteriocidal antibodies are also absent from commercially available gamma globulin preparations that contain only gamma-2 globulins (Cohn Fraction 11).

Newborns may fail to develop hemolytic disease in the presence of maternal anti-Rh antibodies, particularly babies of mothers with "saline-type" Rh agglutinins. It is now known that saline-active agglutinins are 19S globulins, while albumin-blocking antibodies are 7S globulins;[26] it is unlikely that saline-active antibodies would enter the fetal circulation. Immunization with Rh antigen results first in the appearance of the saline antibodies but on continued immunization, the 7S blocking antibodies appear.[27]

About one third of the population has 19S isohemagglutinins, a third has 7S isohemagglutinins, and a third has both the 7S and 19S varieties.[28] Thus, in less than one third of pregnancies is there risk of transmission of substantial titers of isohemagglutinin from the mother to an ABO incompatible fetus. Type O subjects tend to form 7S isohemagglutinins when given A or B substance, in contrast to Type A and B persons who form 19S isohemagglutinins after such stimulation. Thus, a type O motor is more likely to transmit isohemagglutinins to her incompatible infant than a type A or B mother.[29]

PRIMARY PARAPROTEINEMIAS

Plasma cell myeloma, primary macroglobulinemia and $H\gamma^{-2}$ chain disease (Franklin's disease) are currently thought to be neoplasias arising from those cells responsible for the synthesis of the major groups of immunoglobulins, the 7S gamma-2 globulins, the beta-2 globulins, and the 19S macroglobulins. The neoplastic proliferation of the cells producing these gamma globulins results in the elaboration of large quantities of proteins which are physicochemically and immunologically related to their normal gamma globulin counterparts, but are apparently devoid of any function (antibody specificity). A distorted serum electrophoretic pattern is the single common biochemical finding in these patients. All the hyperglobulinemic sera show the presence of a sharp and predominant narrow component, in either the gamma or the beta globulin range (rarely these narrow bands have been found as far forward as the alpha-2 globulins). The globulins of myeloma and Franklin's disease are always ultracentrifugally homogeneous, while those of macroglobulinemia are always heterogeneous. The major component of the macroglobulinemia sera has a sedimentation coefficient of 19S with minor peaks at 7S, 14S, 22S, 26S, and 28S.[30] The presence of the multiple peaks in macroglobulinemia is due to the fact the macroglobulins are polymers of subunits with sedimentation coefficients of 7S and molecular weights of 160,000. However, macroglobulin subunits differ from normal gamma globulin, in that these macroglobulin subunits are held together by disulphide bonds that are disrupted by the addition of sulfhydryl reagents such as cysteine, mercaptoethanol, and penicillamine.

From what is now known of immunoglobulin structure, it is possible to classify the protein abnor-

malities in these primary paraproteinemias in terms of their apparent relationship to the structural subunits of the normal gamma globulins. Neoplastic cells in any given case of plasma cell myeloma may produce a) an excess of free L chains (Bence Jones protein), b) complete gamma globulin molecules with either gamma-2 or beta-2 H chains combined with antigenic type I or type II L chains (so called myeloma protein), or c) complete beta-2 or gamma-2 globulin plus an excess of free L chains. At present, only five cases are known in which an excess of free H polypeptide chains (gamma-2 type) were produced by neoplastic cells without concurrent L chain synthesis.[31 32] The 19S beta-2 macroglobulins in cases of primary macroglobulinemia are composed of beta-2 macroglobulin specific H chains, in combination with L chains of either type I or type II. In addition, 10 to 15 percent of such patients will have urinary L chains as well (Bence-Jones protein).

Until quite recently, the serum and urinary paraproteins produced in cases of myeloma and primary macroglobulinemia, although individually specific, were believed to be homogeneous molecules elaborated by homogeneous populations of neoplastic cells derived from a single neoplastic clone. The multiple peaks on ultracentrifugation displayed by macroglobulins and beta-2 myeloma globulins were known to be due to polymer formation, but this polymer heterogeneity was not incompatible with the one clone — one protein hypothesis. There is now evidence for another kind of heterogeneity (not due to polymer formation) in a large number of murine and human gamma-2 myeloma globulins.[33 34] These studies have shown that the majority of Bence-Jones proteins and gamma-2 myeloma proteins, which give a single peak in conventional electrophoresis, separate into two to five narrow, discrete bands in starch gel electrophoresis, and this narrow-banded electrophoretic heterogeneity is readily distinguishable from the widely separated bands of the polymer type heterogeneity. Whether this narrow-banded heterogeneity is due to different configurational arrangements of polypeptide chains, a situation compatible with the one clone hypothesis, or due to differences in amino acid residues, incompatible apparently with the one clone hypothesis, is yet to be determined. In fact, it is uncertain whether the proteins of myeloma and macroglobulinemia are truly abnormal, or merely normal constituents produced in abnormal amounts as a result of neoplastic proliferation. Though, as yet, no antibody specificity can be attributed to these proteins, the same is true of a large part of the gamma globulin fraction of normal serum.

Franklin's Disease ($H\gamma^{-2}$ chain disease) is an entity recently described by Franklin.[31] Shortly thereafter, Osserman found two more cases in whom the diagnosis was suspected on clinical grounds, and later confirmed by laboratory studies. He found an additional two in whom the diagnosis was made retrospectively, after a search of his files for similar patients. The clinical features are discussed in the original papers.[31 32] Most distinctive is the unique abnormality in protein metabolism which consists of the production of a great excess of H polypeptide chains of gamma-2 globulin. There is also proteinuria which is Bence-Jones negative, due to an electrophoretically homogeneous protein of fast gamma mobility. Serum electrophoresis shows an abnormal peak, in each case corresponding to the abnormal urinary protein in electrophoretic mobility. The molecular weight of 53,000 accounts for its retention in serum in excess of Bence-Jones protein, in patients with myeloma and Bence Jones proteinuria. The fact that the polypeptide chains in these cases are all related to gamma-2 globulin, and unrelated to either macroglobulins or beta-2 globulins, raises the question as to whether future studies might disclose cases of H^{b-2} globulin and H^{b-2m} chain disease. To date, unlike free L polypeptide chains (Bence-Jones protein), free H polypeptide chains have not been detected in normal subjects.

PARAMYLOIDOSIS

The tissue infiltrates that occur in secondary amyloid appear to arise *in situ* from the reticuloendothelial cells of parenchymal organs and lymph nodes. Initially, proliferation of reticulum cells occurs; these differentiate along *plasmacytic* lines, accumulate PAS positive material in their cytoplasm, and finally degenerate with shrinkage and pyknosis of nuclei and separation of their cytoplasmic PAS-positive amorphous aggregates.[35 36] These intracellular aggregates are chemically and immunologically similar to gamma globulin. The *paramyloid* deposits that develop in association with overt plasma cell myeloma, and the related (if not identical) condition of primary amyloid, have a different distribution and different tinctorial properties. Apitz has found an increased number of marrow plasma cells in all cases he examined of primary amyloidosis.[37] It is known that the tissue infiltrates that develop in association with overt multiple myeloma, can precede the skeletal manifestations by months or years, and, indeed, these infiltrates may kill the patient through cardiac decompensation or malnutrition, before gross bone destruction has occurred. In cases of this type, abnormal plasma cell proliferation would be detected only if carefully sought for in repeated marrow aspirations, or in careful post mortem examination of multiple skeletal sites. Many of these patients will exhibit Bence-Jones proteinuria with hyperglobulinemia, and in twelve patients with primary amyloidosis studied by Osserman,[38] six never had overt evidence of skeletal destruction. In the remaining six who did, destruction preceded the infiltrates in one, arose concomitantly in one, and followed the appearance of the tissue infiltrates in four cases. In all of these twelve patients, electrophoresis of urine demonstrated characteristic urinary proteins (Bence-Jones protein) to be present. Again, in all these twelve cases of primary amyloidosis, abnormal numbers of plasma cells were documented by bone marrow aspiration, post mortem examination, or both. Kyle and

Bayrd[39] reviewed the data on eighty-one patients with primary amyloidosis. Proteinuria was present in 91 per cent, and Bence-Jones proteinuria in 29 per cent. Electrophoresis of the sera of 25 patients was performed, and revealed patterns diagnostic of myeloma in seven, hypogammaglobulinemia in ten, and abnormal, yet not diagnostic, patterns in seven others; only one had a normal pattern. These same authors note that, although primary amyloid and amyloid associated with myeloma are functionally alike, found in the same sites, and present the same clinical picture and histologic features, they have been separated traditionally on what seems a solely negative observation, namely the lack of a sufficiently diagnostic marrow to satisfy the observer's criteria for a diagnosis of myeloma. Diagnosis is often difficult in clinically clear cases as the marrow may appear deceptively mature, deficient in distinctive cells, or be confused with lymphocytic processes. It should not be remarkable if the same were found to be true of patients manifesting systemic amyloidosis.

Osserman and Talpatsuki[40] suggest that the Bence-Jones proteins in cases of this type (myeloma with paramyloidosis) may have a particular affinity to bind certain tissue constituents, either tissue proteins or polysaccharides. This may be the mechanism for formation of these insoluble proteinaceous infiltrates, and would be consistent with the fact that these deposits are composed, at least in part, of protein which is antigenically related to gamma globulin. In other words, they have diffused through the capillary beds of few, if any, tissues and in the extravascular spaces of only certain sites do they find their complementary polysaccharides with which they can form insoluble complexes.

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REFERENCES

1. The Plasma Proteins, ed. Putnam; Academic Press. New York, London, 1960.
2. Tiselius and Kabat; J. Exp. Med. 69: 119, 1939.
3. F. S. Rosen; New England J. Med. 267: 491-497: Sept. 6, 1962.
4. Fahey and Lawrence; J. Immunol. 91: 594, 1963.
5. Porter, R. R.; The Plasma Proteins, Vol. 1. Edited by F. W. Putnam. Academic Press, New York.
6. Petermann, M. L.; The Plasma Proteins. Edited by F. W. Putnam, Vol. 2; Academic Press Inc. New York, 1960.
7. Porter, R. R.; British Med. Bull., 19: 3, 1963.
8. Franklin, E. C.; The Immune Globulins in Man. The Pediatric Clinics of North America. W. B. Saunders Co., Nov. 1963.
9. Mannik and Kunkel; The Immunoglobulins. Bulletin on Rheumatic Diseases, XIII: 8, April 1963.
10. Porter, R. R.; In Gellham and Hirschberg W. Basic Problems in Neoplastic Disease. Columbia University Press, New York and London, 1962.
11. Cohen; Nature, Lond., 197: 253, 1963.
12. Harboe, Osterland, and Kunkel; Science 136: 979, 1962. 1962.
13. Franklin, Fudenberg, Meltzer, and Stanworth; Proc. Nat. Acad. Sci. Wash., 48: 914, 1962.
14. Franklin, E. C.; The Immune Globulins. Arthritis and Rheumatism, 6: 4, August 1963.
15. Cohen, S.; British Med. Bulletin, 19: 3, 1963.
16. Cohen and Freeman; Bioch. J., 76: 475, 1960.
17. Gross, Gitlin, and Janeway; New Eng. J. Med., 260: 121, 1959.
18. Ogryzlo, Maclachlan, Dauphinee, and Fletcher; Am. J. Med., 27: 4, 1959.
19. Cohen and McGregor; in Garnham and Reitt, Immunity to Protozoa; A Symposium of the British Society for Immunology. Blackwell, Oxford, 1963.
20. Cecil and Loeb; Textbook of Medicine, ed. Beeson and McDermott. W. B. Saunders Co., Phil. and London, 1963.
21. Prasad, Aboud, Salowi, and Schubert; Ann. Int. Med., 61: 2, Aug. 1964.
22. Abruzzo and Christian; J. Exper. Med., 114: 1961.
23. Kunkel; The Plasma Proteins. Vol. 1. Ed., Putnam; Academic Press, New York, London, 1960.
24. Fudenberg and Martensson; Bulletin on Rheumatic Disease, XIII: 9, May 1963.
25. Clinical Staff Conference; Ann. Inter. Med., 61: 3, Sept. 1964.
26. Chan and Deutsch; J. Immunol., 85: 1960.
27. Abelson and Rawson; J. Immunol., 83: 1959.
28. Rawson and Abelson; J. Immunol., 82: 1959.
29. Rawson and Abelson; J. Immunol., 85: 636-639, 1960. Ibid., 85: 640-649, 1960.
30. Putnam, F. W.; New Engl. J. Med., 261: Oct. 29, 1959.
31. Franklin, Lowenstein, Bigelow, and Meltzer; Am. J. Med., 37: 3, 1964.
32. Osserman and Takatsuki; Am. J. Med., 37: 3, 1964.
33. Fahey; J. Clin Invest., 42: 1, 1963.
34. Askonas; Biochem. J., 79: 33, 1961.
35. Teilum; Am. J. Path., 32: 945-959, 1956.
36. Vasquez and Dixon; J. Exper. Med., 104: 727-737, 1956.
37. Cited in Osserman; New Engl. J. Med., 261: 952-960, 1006-1014, Nov. 5 and Nov. 12, 1959.
38. Osserman; New. Engl. J. Med., 261: Nov. 5 and Nov. 12, 1959.
39. Kyle and Baird; Arch. Int. Med., 107: 344-353, March 1961.
40. Osserman and Takatsuki; Medicine, 112: 6, November 1963.

Autoimmunity and Hypersensitivity

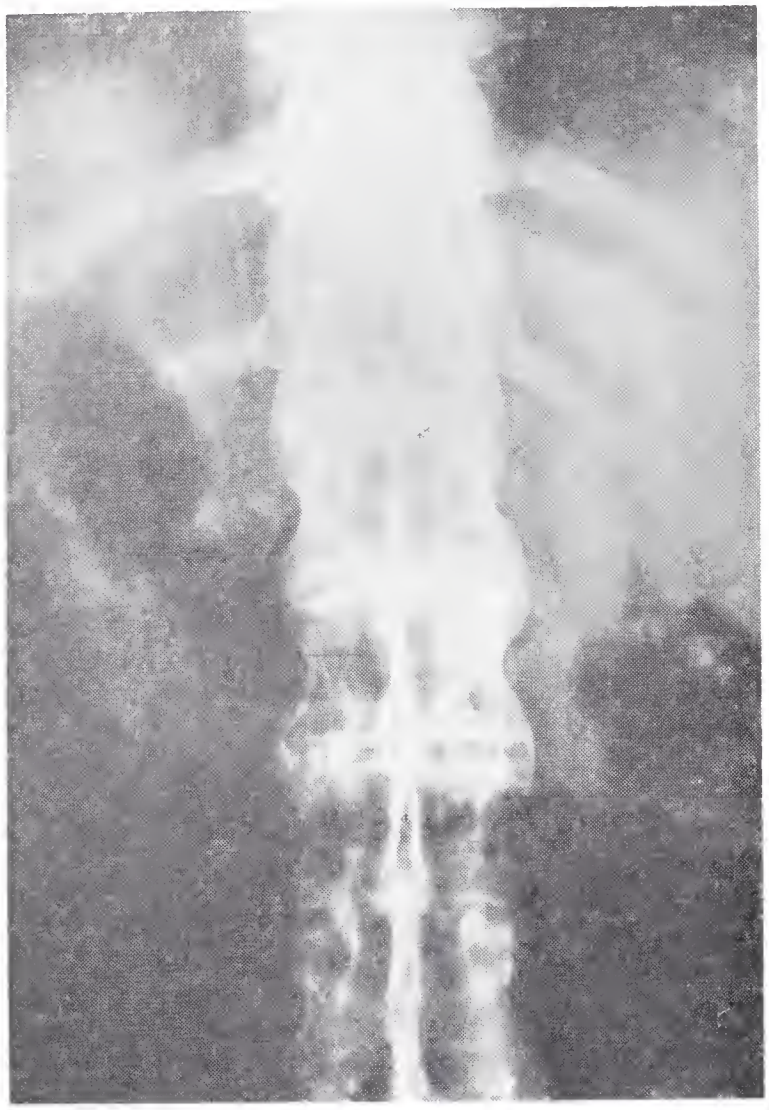

Figure 1.

a) Of what condition is this X-Ray typical?
b) What bony changes occur in the disease?
c) How does this disease present? What complications may occur?

Figure 3.

a) Identify this lesion.
b) How does the pathological picture of this lesion differ from that in rheumatic fever?
c) What are the non-articular complications of this disease?

Figure 2.

a) What disease is associated with this lesion? What other finger changes occur in this disease? What other "autoimmune" conditions could give a similar inflammation at the base of the nail?
b) What other skin manifestations are there of this disease?

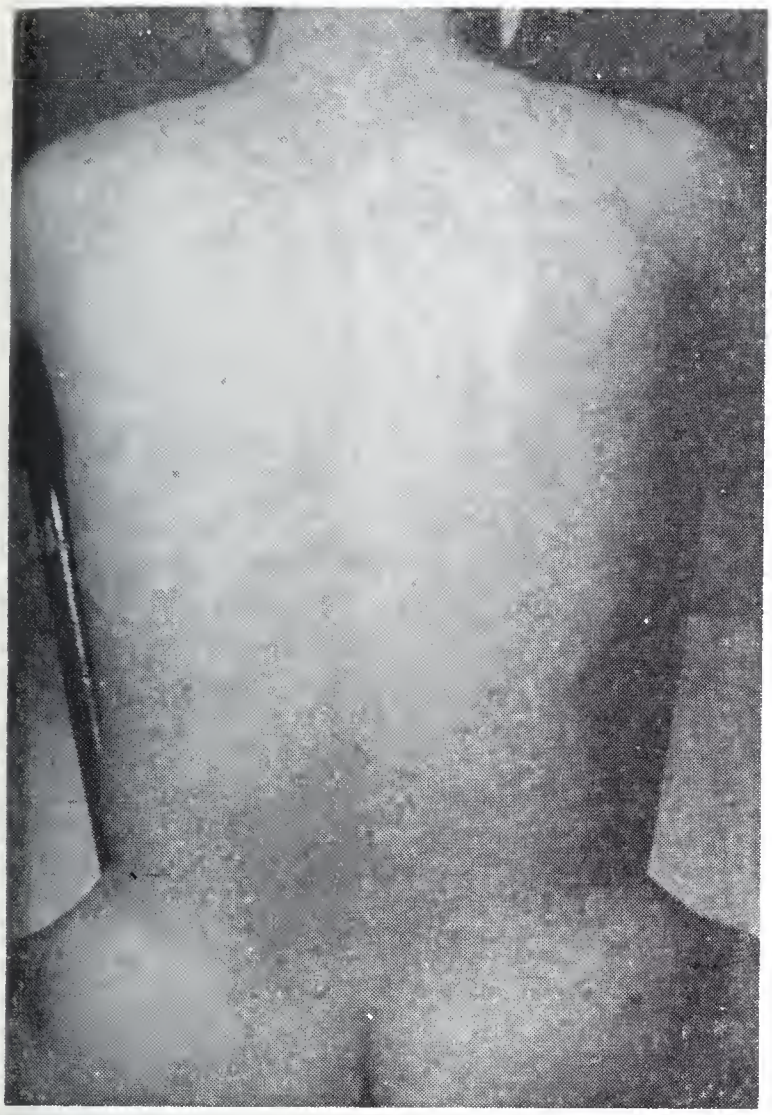

Figure 4.

Figure 5.

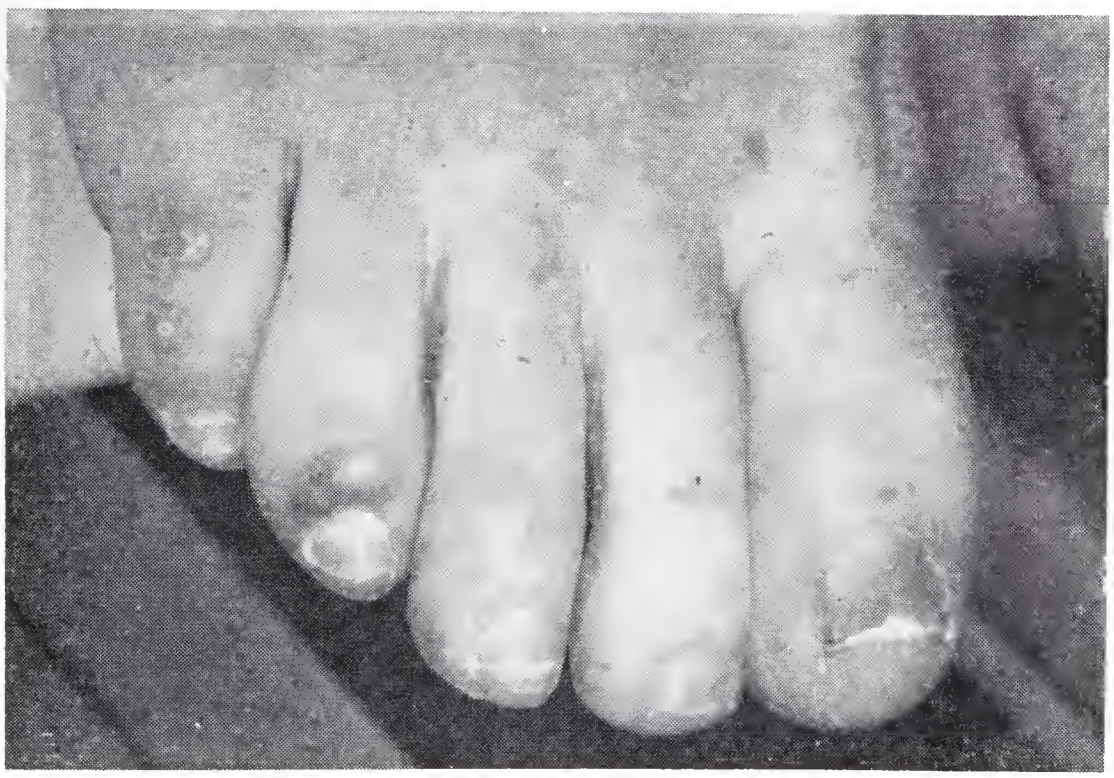

Figure 6.

a) What is the aetiology of the above skin lesions?

b) Using penicillin as an example, comment on skin testing in drug allergy.

Answers on page 170.

Burnet

The field of immunology has become the source of considerable interest for medical scientists and research workers. The beneficial aspects of the immune phenomenon were well described many centuries ago when a King, named Mithridates, whose interests centred around poisons, was able to immunize himself to all the known poisons of his time. In 1789, Jenner observed that dairymaids who contracted cowpox did not become infected with smallpox. Many other studies by Pasteur, Ehrlich, etc., showed that protective substances seemed to develop after exposure to toxic substances and were of great significance in the survival of an organism. In 1902, Portier and Richet showed that immunity could have in contrast, deliterious effects upon an organism.

For many years the study of immunology was shuffled into the background, for many considered that the more essential aspects of immunity had been well documented. Revived interest was sparked by the observations of Landsteiner and his group concerning the Rh antigen system. Another variant of immunologic process made its appearance, autoimmunization. Many diseases today are described as being autoimmune, to which pernicious anemia has been tentatively added. Many varied aspects of this phenomena have been subjected to extensive medical research. In similar fashion, many different theories of the mechanism of autoimmunization have been proposed and one of recent note is that of Burnet's *clonal selection theory*. His concepts of "self" recognition and forbidden clones have paved the way for research into the possibility of disturbances in the antibody forming apparatus causing the cells to synthesize antibody against normal components of the organism. The discovery of acquired immunological tolerance by Burnet and Medawar has undoubtedly opened up new fields of study.

Frank MacFarlane Burnet was born on September 3, 1899, at Traialgon, Australia. He received his primary education at a local state school in Terang and at the age of thirteen, won a scholarship to Geelong College, Melbourne.

The Great World War made Burnet decide that medicine was the more important profession and in 1916, he enrolled at the Melbourne University Medical School. After graduation, he joined the Royal Melbourne Hospital and was offered in 1923, the post of resident pathologist. The director of the Hall Institute, Dr. C. H. Kellaway, convinced Burnet to visit Britain in order to expand his experience in research. Being offered a research job in the Lister Institute, he began his research of the bacteriophage phenomenon.

He visited England in 1932, after a brief return to Australia in 1928, to take up the post of assistant director at the Walter & Eliza Hall Institute. He developed a technique for cultivating viruses in living chick embryos and was associated with the discovery of the influenza virus.

His return to Australia in 1934 was to be permanent, and later he became the director of the Hall Institute. His studies at this time still centred around the problem of bacteriophages and viruses. In 1942, he was elected a Fellow of the Royal Society and was later awarded the Society's Royal Medal for his contributions to medical knowledge.

Developing out his studies in virology was the immune phenomenon. His interest progressively shifted to the study of immunology. The problems of graft reactions and autoimmune diseases captivated his interest. His theories on "self" recognition paved the way to the writing of his book on the clonal selection theory of acquired immunity. His work on immunity brought to Burnet many honours. He was Knighted in 1951, received the Order Of Merit in 1958, the Copley Medal in 1959, and together with Medawar in 1960, the Nobel Prize for Medicine.

A.G.L.

Myasthenia Gravis and the Thymus

"Where there is much desire to learn there of necessity will be much arguing, much writing and many opinions: for opinion in good men is but knowledge in the making."

—JOHN MILTON, *1644.*

INTRODUCTION

It is most challenging to find evidence of thymic abnormality in clinical and pathological conditions associated with disturbed immunological function. One of the best known of these clinical conditions is myasthenia gravis, a disease reasonably well defined clinically, in which there is a failure of normal function of the myoneural apparatus. It is a chronic disease characterized by a variable weakness following the use of voluntary muscles, particularly those subserving ocular movement, facial expression, mastication, deglutition and respiration. Cardiac and smooth muscle are not affected. At first the weakness takes the form of a failure of contraction after any maintained effort but in more severe phases of the disorder, the muscles first and most severely affected cease to contract even after prolonged rest.

Part I: Immunological Considerations

E. G. Bell Ph.D., 6T5

For many years there has been clinical and pathologic evidence of association of myasthenia gravis with benign thymoma. Recent studies place its incidence at about 15% of myasthenia gravis patients.[1] Whether the thymus is enlarged or not, evidence is regularly given that the organ may be making an immunologic response is myasthenia gravis not seen in the normal thymus. In some studies, as many as 85% of myasthenia patients have demonstrable morphologic abnormalities of the thymus.

On the basis of a detailed analysis of 446 cases and of the literature, Simpson[2] first advanced the hypothesis that myasthenia gravis is a manifestation of an auto-immune disorder in which antibody is produced against neuromuscular end-plate protein; this antibody was assumed to compete with acetylcholine in the end-plate, thus causing an abnormal effect on normal neuromuscular transmission. Simpson felt that the muscle protein may be rendered antigenically foreign by the same type of association with upper respiratory tract infection as is believed to occur in rheumatic disorders and acute nephritis. He also speculated that a posterior pituitary factor might predispose to this by disturbing cell membrane integrity. Indeed, if protein which was normally confined within the muscle cell were allowed to escape, the auto-immune reaction could be initiated.

Marshall and White,[3,4] in exploring the immunological reactivity of the thymus gland, observed that the direct injection of antigen into the thymus of guinea pigs led to the formation of germinal centers, plasma cells and the production of antibody. The failure of these thymic changes to occur with parenterally administered antigen, other than by direct injection, suggested that there was either a barrier against the entry of the antigen into the thymus or a lack of a suitable phagocytic mechanism to segregate it into the gland. Subsequent experiments proved that there was rapid phagocytosis of both vital dye and antigenic material following local trauma; this suggested that a barrier exists between the blood and thymic parenchyma. They concluded, therefore, that there can be no reaction between the intact thymus and circulating antigen and, as a result, no antibody formation in the gland. These workers agreed with Simpson's hypothesis of an auto-immune basis, but not with the statement that a motor end-plate protein, or other muscle tissue antigen, is primarily involved, in view of the existence of the blood barrier. They offered as a means of explanation, Burnet's concept[5] that there is a proliferation of a forbidden clone capable of leading to antibody formation[6] or reacting immunologically against nerve endings or muscle components. If it is accepted that there is a blood barrier shielding the thymus against circulating protein, then it would appear a particularly favorable site for such clonal growth to take place, as contact with antigen is a mechanism postulated by Burnet for the destruction of such forbidden clones which are capable of an immune response against an individual's own tissues. Some doubt has been cast on the existence of a blood-thymic barrier by the recent work of Sainte-Marie,[7] in which he showed thymic changes by intramediastinal injections of bovine serum albumin; the controversy at present is unresolved.

Nastuk and his associates searched for a blood circulating agent[8] which could cause a deficit in neuromuscular transmission by producing a curare-like ef-

fect. They measured changes in muscle tension output of an indirectly stimulated frog sartorius muscle-sciatic nerve preparation. It was found that the serum from some myasthenia patients led to a reduction in muscle tension output; this appeared to parallel an observed cytolytic destruction of fibers lying on the surface of the sartorius muscle used in the assay. Impressed by Simpson's concepts of an immune basis for the disease[2] and the known essential part that serum complement (C_1) plays in immune hemolysis,[10] they carried out determinations on complement activity in the serum of myasthenia patients.[9] They found that the serum complement in myasthenia gravis fluctuated over an abnormally wide range; they felt that this was related to exacerbations and remissions of the disease. They visalized the change in serum complement by participation in an auto-immune response, as follows: The myasthenia gravis patient develops an auto-immunity against one of the components (M) of the skeletal muscle fibers. M, an entity in the plasma membrane or an intracellular muscle element, combines with a foreign antigen (S) and the combination MS being antigenic leads to the formation of antibodies (A) against it. On reaching the muscle fibers, A would be bound, leading to the formation of MSA which in turn would bind serum complement. Therefore, serum complement would be consumed and its presence would lead to either cytolytic destruction of the cell membrane or a subcytolytic alteration in the configuration of the cell membrane. This could lead to a decreased acetylcholine sensitivity of post-junctional membrane, inhibition of the cell membrane conduction, or failure of excitation-contraction coupling. It was also speculated that the muscle fibers were either reversibly or irreversibly damaged and that the terminal arbors of the motor nerve were directly or indirectly involved.

Additional support of an auto-immune basis came from work of Strauss and co-workers[11] using immunofluorescence techniques.[12] They showed that there was a muscle binding, complement-fixing component in the crude globulin fraction of myasthenia sera. This component could not be demonstrated in similarly prepared normal serum globulin. Untagged myasthenia gravis globulin was shown to competively block adherence of fluorescein-tagged myasthenia globulin to skeletal muscle striations. Normal sera globulins did not block the effect. It was also illustrated that the myasthenia globulin-fraction fixed guinea pig complement to human skeletal muscle, whereas normal human serum globulin failed to fix complement by this procedure.

In 1962, Beutner and his associates[13] began studying myasthenia sera in an attempt to gain a better understanding of the component of the affected sera described by Strauss.[11] Beutner directed his experiments towards determining the specificity and the iso-or auto-antibody nature of the component. He confirmed Strauss' observations that there was a factor in the myasthenia sera with specific affinity for skeletal muscle and heart muscle. He concluded that the factor was an auto-antibody with a high degree of specificity towards skeletal muscle. Beutner felt that the mere demonstration of circulating auto-antibodies in spite of exquisite specificity was not sufficient proof for the auto-immune cause of the disease. He stated that additional evidence should be furnished by reproducing the disease in experimental animals by active immunization similar to studies of experimental auto-immune thyroiditis.

Many investigators have shown *in vitro* localization of gamma globulin in the alternate striations of skeletal muscle. However, no one has demonstrated *in vivo* localization of gomma globulin in muscle biopsies taken from myasthenia patients. Indeed, very little work of this nature is reported in the literature; the emphasis has been placed on offering additional proof of Strauss' and Beutner's observations of *in vitro* fixation. Additional evidence for this work was given by Feltkamp's study[14] on the nature of fluorescence seen using an indirect fluorescent antibody technique. Feltkamp found that there were three types of recognizable fluorescence: one in which only the sarcolemma was involved, a situation also found in preparations using normal sera; another in which there was fluorescence of the muscle fiber; and finally one in which there was a zebra type of fluorescence. These latter observations were found only in preparations made using myasthenia sera. Forty-two percent of Feltkamp's patients with myasthenia gravis exhibited the muscle fiber and zebra type of fluorescence, whereas none of the normal controls showed the phenomena.

The preceding experimental evidence plus the work of Miller[15] in showing that the thymus has an immunologic function, prompted Van der Geld[16 17 18] to search for anti-muscle and anti-thymus antibodies in myasthenia sera. He used the antiglobulin consumption test,[16] Feltkamp's modification of Coons' indirect immunofluorescent technique,[14] and Boyden's hemagglutination technique with tanned erythrocytes,[19] to investigate the phenomena. In his investigations he found antibodies against skeletal muscle, thymus and thyroid tissue in a study on the sera of over 100 patients with myasthenia gravis. Cross antibody studies suggested two antibodies; one against muscle tissue only, and other against muscle and thymus tissue. This gave rise to speculation that there were two antigenic determinants involved in muscle tissue which evoked an antibody response; one of these antigenic determinants was common to one found in thymus tissue. Several interesting facts emerged from his investigations: Patients whose myasthenia was associated with a thymoma had a higher incidence of anti-muscle antibodies and their clinical condition was more severe; patients with ocular myasthenia only, had a lower incidence of anti-muscle antibodies and finally, anti-muscle and anti-thymus antibodies were demonstrated after apparent total thymectomy.

Studies on patients suffering from chronic non-specific thyroiditis revealed that the tanned cell hemagglutination technique adapted for the detection of thyroglobulin by Witebsky and Rose[20] provided the most sensitive method for detecting thyroglobulin auto-antibodies. Djanian[21] used this technique to demonstrate muscle antibodies in myasthenia sera. Both skeletal and heart muscle preparations served as coating antigens under appropriate conditions; cells coated with smooth muscle failed to react. These observations suggested that skeletal and heart muscle antibodies are operative in the tanned cell hemagglutination test. Tanned red cells, coated with fractions prepared from muscle of each of the antibody-producing patients, reacted with the patient's own sera pointing to the auto-antibody nature of the reaction. Comparative tests were performed with the tanned cell hemagglutination test, immunofluorescent staining and complement fixation. Djanian concluded that the hemagglutination reaction was at least as sensitive and specific as the immunofluorscent stains in detecting the antibodies, but was not as highly specific as was reported in studies for the detection of thyroglobulin auto-antibodies.

The widescale use of immunofluorescent reactions by White and Marshall[22], Feltkamp[23], Strauss et al [4], has shown that the serum of patients with myasthenia gravis contains an anti-nuclear factor (ANF) or factors which are gamma globulins and which bind with antibody-like specificity to somatic cell nuclei. At present the anti-nuclear factor is best regarded as a 'marker' for a possible auto-immune disease without being in itself of pathogenic significance. The relationship between the presence of anti-nuclear factors in myasthenia gravis and various collagen diseases was studied by several investigators. White and Marshall[22] demonstrated an anti-nuclear factor in the sera of 6 of 16 patients with myasthenia gravis using nuclei of thyroid tissue. Of these 6, one had systemic lupus erythematosus, one had cutaneous lupus and one had rheumatoid arthritis. Feltkamp,[23] using an indirect fluorescent antibody technique on blood smears, observed nuclear fixation in 18 of 103 patients with myasthenia gravis; all 18 were negative to an indirect LE cell test. Of the 103 patients, 6 had rheumatoid arthritis; one of the 6 showed anti-nuclear fixation.

In attempting to determine what significance should be placed upon the evidence cited in this paper, it is necessary to have some standards by which to assess the credibility of an auto-immune basis for a disease entity. In 1958, Witebsky[25] advanced certain postulates which he felt must be satisfied in order to correctly relate pathological changes to the demonstration of auto-immune phenomena. He stated: i) that it should be possible either to demonstrate circulating antibody active at body temperature in serum of patients suffering from the disease, or to demonstrate cell bound antibody by indirect means; ii) that antigen, against which the antibody is directed, should be characterized or even isolated; iii) that antibodies should be produced against the same antigen in experimental animals; and iv) that pathological changes that are similar or identical with those seen in human disease, should appear in the corresponding tissue of an actively sensitized animal.

The attempts of most of the investigations reported in this paper have been directed towards demonstrating antibody fixation by certain tissues, complement fixation, complement consumption and the presence of an anti-nuclear factor. The circulating antibody detected by many investigators has *not* been demonstrated to be the cause, rather than the result, of the disorder, despite its exquisite specificity for muscle tissue. The situation may be similar to the development of myoglobulinuria after cardiac surgery[26] or to the appearance of anti-heart antibodies after myocardial infarction.[27] The recent statement of Sir MacFarlane Burnet[28] that myasthenia gravis is unquestionably an auto-immune disease, should not stampede the student into believing that the problem has been resolved. The hypothesis that the disease has an auto-immune basis has not been proven despite the considerable research effort of the past five years. A more logical analysis of the situation was made by Strauss,[29] who stated that none of the evidence referenced in the literature directly supports the point of view that myasthenia gravis has auto-immune etiology or pathogenesis. The only acceptable conclusion that may be drawn now, is that myasthenia gravis is associated with disturbed immunological function, but is not necessarily an auto-allergic or auto-destructive process.

While it is true that caution should always be exercised in accepting any statement on the basis of indefinite evidence, the converse is also true. Therefore, the available evidence cited in the literature should not b discarded, but should be used to encourage further work. Perhaps it will be from consideration of these papers that the critical experiment will be designed.

REFERENCES

1. **Viets, H. R. and R. S. Schwab: Thymectomy for Myasthenia Gravis, Springfield, Ill.; Thomas, (1960).**
2. **Simpson, J. A.: Scot. Med. J. 5: 419, (1960).**
3. **Marshall, A. H. E. and R. G. White: Brit. J. Exp. Path. 42: 379, (1961).**
4. **Marshall, A. H. E. and R. G. White: Lancet 1: 1030, (1961).**
5. **Burnet, F. M.: Clonal Selection Theory of Acquired Immunity, P. 135, Vanderbilt Univ. Press., (1959).**
6. **White, R. G.: Proc. R. Soc. Med. 50: 953, (1957).**
7. **Sainte-Marie, G.: J. of Immunology, 91: 840, (1963).**
8. **Nastuk, W. L.: Am. J. Med. 26: 394, (1959).**
9. **Nastuk, W. L. et al: Proc. Soc. Exp. Biol. and Med. 105: 177, (1960).**
10. **Lepow, I. H.: Chapter 21, Mechanisms of Hypersensitivity; M. W. Chase, Editor. Little, Brown and Company, (1959).**
11. **Strauss, A. J. L. et al: Proc. Soc. Exp. Biol. and Med. 105: 184, (1960).**

12. Coons, A. H. and M. H. Kaplan: J. Exp. Med. 91: 1, (1950).
13. Beutner, E. H. et al: J. A. M. A. 182: 46, (1962).
14. Feltkamp, T. E. W. et al: Vox Sang 8: 317, (1963).
15. Miller, J. F. A. P.: Lancet 2: 748, (1961).
16. Van der Geld, H. and H. J. G. H. Oosterhuis: Vox Sang 8: 196, (1963).
17. Van der Geld, H. et al: Proc. Soc. Exp. Biol. and Med. 115: 782. (1964).
18. Van der Geld, H. et al: Lancet 2: 373, (1963).
19. Boyden, S. V.: J. Exp. Med. 93: 107, (1951).
20. Whitebsky, E. and N. R. Rose: J. of Immunology, 76: 408, (1956.)
21. Djanian, A. Y. et al: J. Lab. and Clin. Med. 63: 60. (1964).
22. White, R. G. and A. H. E. Marshall: Lancet, P. 120, July 21, 1962.
23. Feltkamp, T. E. W.: Lancet 1: 667, (1963).
24. Strauss, A. J. L. et al: Lancet, p. 351, July 21, 1962.
25. Witebsky, E.: Immunopathology, 1st International Symposium, 1958. Grabar, P. and P. Miescher, Editors. Benno Schwabe, Basel and Stuttgart.
26. Kaplan, M. H. et al: J. Exp. Med. 113: 17, (1961).
27. Resnik, W. H. and T. R. Harrison: Principles of Internal Medicine, Chap. 256, Vol. II. Harrison, T. R. et al, Editors. McGraw-Hill, (1962).
28. Burnet, F. M.: Brit. Med. J. 5308: 807, (1962).
29. Strauss, A. J. L.: Brit. Med. J. 5322: 56, (1963).

Part II: Pathology of the Thymus in Myasthenia Gravis

B. Zimmerman 6T5

The thymus is a pink-grey bilobed mass that weighs about 30-40 grams at puberty but normally involutes after this time.

Microscopically the lobes of the gland are divided into incomplete lobules by septa extending inward from a thin capsule. The gland is composed of lymphoepithelial tissue on a reticulum back-ground. The lymphocytes are distributed heaviest towards the periphery of the lobule (cortex) and more thinly centrally (medulla). Hassal's corpuscles are numerous in the medulla. These consist of a few concentric layers of epithelial cells with a pyknotic centre.

Clark, in 1963,[1] described the electron microscopic structure of the mouse thymus. He found the basic structure was a packet of lymphocytes enclosed completely by epithelial cells. These latter had desmosomes scattered along the tightly knit adjacent cell membranes. The completeness of the epithelial fence is suggested as the possible barrier to circulating antigens.

Pathology

The known pathological relation of the thymus in myasthenia is predominantly statistical.[2,3] In 15-20% of cases, there is a thymoma. In 50-75% there is thymic hyperplasia, and in the rest, the thymus appears normal. The thymomas are classified according to their predominant cell type; that is, squamoid, lymphoid, spindle cell, epithelial, etc. The thymomas associated with myasthenia are most often predominantly lymphoid.

But it is the histology of the non-tumourous hyperplasia which is the most interesting. These tend to cortical hyperplasia and increased medullary lymphocytes with the presence of germinal centres. These germinal centres are rare in the normal thymus. Burnet[4] feels they have the same significance as those in lymph nodes — that is, immunological. It has been shown in lymph nodes by experimental and immunofluorescent techniques on nodes of active Rheumatoid Arthritis[5] that the stimulus for the formation of germinal centres is antigenic. Evidence seems to be that germinal centres represent a multiplying clone of immunologically competent cells initiated by a cell reacting to some antigenic stimulus. Burnet[4] has modified his clonal selection theory to include these new concepts and feels that forbidden clones have their origin in the thymus and result from mutation within the gland. The normal thymus plays an important role in preventing the emergence of forbidden clones.

Clinically, myasthenia has a statistical relationship with other autoimmune diseases.[6] A frequent associate of myasthenia and thymoma is thyroiditis. Polymyositis, dermatomyositis, disseminated lupus erythematosus, anemias, and hypogammaglobulinemias all have been associated with thymic changes and myasthenia gravis. In fact, disseminated lupus erythematosus has been found with thymic changes and germinal centres similar to that found in myasthenia, but without this latter disease.

Experimentally, conflicting results have been reported about the reproduction of germinal centres in guinea pig, rat and mice thymuses by direct innoculation of antigen into the gland. Marshall & White[7] (1961) created germinal centres with direct injection of paratyphoid-typhoid vaccine, whereas, more recently Blau and Waksman[8], using bovine serum albumin, were unable to obtain these results.

Other experimental work has included the use of New Zealand Black mice.[9] These are an "autoimmune strain" of mouse developed through inbreeding. They are highly susceptible to the development of hemolytic disease with a positive direct Coombs. They also demonstrate other circulating antibodies, with frequently positive antinuclear factor tests, and occasionally L.E. cells. The terminal phase of this autoimmune process is the development of a severe type of glomerulo-nephritis. The most important point about these mice is that at 3 to 9 months of age, lesions develop in the thymus which have all the characteristics of germinal centres. These centres are also found in the lymph nodes and spleen. The significance again seems to be clones of immunologically competent cells, multiplying in the thymus where they are normally forbidden.

Another recent paper by the Russian worker Svet-Moldavsky[10] postulates some known or unknown pituitary hormone with a thymotropic function. He designed an experiment on the basis that pituitary, implanted directly into the thymus of animals, should cause a drastic activation and folicular production. Using non-inbred albino rats, he implanted anterior pituitary fragments into the thymus of each animal. More than 75% (22 of 27) on the 14th to 60th day afterwards became depressed, constrained and motionless when set on the edge of a table, drooping their heads for hours. Aspiration was enforced. Electrophysiological studies demonstrated a neuro-muscular disorder. There were also changes in serum proteins, and anemia. Control animals injected with brain, posterior pituitary, and other substances did not show this effect.

At autopsy, the thymuses of these mice were almost normal in appearance, but there was marked germinal centre formation in lymph nodes and spleen.

REFERENCES

1. **Clark; Amer. J. of Anat. 112: 1, 1963.**
2. **Harvey and Johns; Am. J. of Med. 32: 1, 1962.**
3. **Lattes; Cancer 15: 1224, 1962.**
4. **Burnet; Australasian Ann. Med. 11: 79, 1962.**
5. **Congdon and Goodman; Quoted by Burnet and Holmes; Ann. of Int. Med. 59: 265, 1963.**
6. **Mayo Clin. Proceedings; Vol. 39, No. 1, 1964.**
7. **Marshall and White; Lancet 1: 1030, 1961.**
8. **Blau and Waksman; Immunology 7: 33, 1964.**
9. **Burnet; J. Path. and Bact. 88: 229, 1964.**
10. **Svet-Moldavsky; Nature 203: 4964, 1964.**

Part III: Pathophysiological Considerations

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There are, at present, few authorities on myasthenia gravis, who do not consider it to be a disease of an autoimmune nature. Yet, in the early part of this decade, medical literature contained only a small number of articles concerned with the possible autoimmune aspects of myasthenia.

There is a good reason for this rather radical change in outlook. Although the pathological linkage of the thymus and myasthenia gravis began in 1901, with the work of Weiger,[1] it has only been in the last few years that the normal function of the thymus has begun to be elucidated, and its role in immunology appreciated.

Function of the Thymus Gland

What, then, is known about the role of the normal thymus? There is a growing body of evidence that the specific function of the thymus is to initiate, during development, the production of lymphocytes, and the ability to react immunologically.[2] Neonatal thymectomy of the experimental animal has been shown in the work of Miller[3], Archer et al[4], Good et al[5], and others, to be associated with failure of development of the lymphoid system, and severe defects in both the cellular and humoral immune response. These thymectomized neonates do not gain weight satisfactorily, they develop a wasting syndrome, they will not reject skin grafts, and they cannot produce antibodies in response to foreign antigens such as typhoid bacilli.

Much work has also been done in an attempt to elucidate the function of the mature thymus. If a mature animal is given ionizing radiation, sufficient to destroy its peripheral lymphoid tissues and immunological mechanisms, complete recovery will not occur if the thymus gland has been removed. However, in mature animals who were not irradiated, the absence of thymus was shown to give rise to no significant immunological defects, presumably because the peripheral lymphoid tissues and immunological mechanisms were already adequately developed.[6 7 8] On the basis of these findings, it would be tempting to conclude that, normally, the mature thymus is functionless. The recent work of Cross and Miller[9] has, however, shown that in the normal adult mouse, immunologically competent cells are present in abundance in the spleen, but are scarce

in the bone marrow. The latter contains instead many lymphoid precursor cells whose descendants can become immunologically competent only in the presence of a functioning thymus mechanism.

The precise identity of the method by which the thymus exerts its control on lymphoid tissue and immunological reactivity has been the subject of many recent investigations.

For the thymus to carry out this function, only two mechanisms seem logical. First, cells possessing immunological competence, or the potential to develop it, may migrate from the thymus to populate the peripheral lymphoid tissues. Secondly, the thymus may release a humoral factor responsible for regulating the production and maturation of immunologically competent cells. Burnet, in 1962, advocated the first mechanism.[10] He postulated that the thymus is the chief first level immunological organ in which lymphoid cells arise. In the second level immunological organs such as spleen and lymph nodes, these cells proliferate to give rise to functionally active descendants. Evidence for this concept has recently been offered by Yunis et al, who have shown that the administration of isogenic (isologous) or allogenic (homologous) thymocytes will reconstitute the immunological capacity of neonatally thymectomized mice.[11]

Evidence for the second possible regulatory mechanism, a thymic hormone, has also begun to accumulate recently. Osoba and Miller[12] implanted embryonic or neonatal thymic tissue, enclosed in Millipore diffusion chambers, in neonatally thymectomized mice. This procedure restored the mice to normalcy in all respects, with the exception that there was some decrease in the lymphocyte population of both blood and tissue. When they examined the implanted thymic tissue after one to two months, they found only epithelial-reticular cells, and no lymphoid cells. They, therefore, concluded that the thymus epithelial-reticular complex produced a humoral factor responsible for endowing lymphoid cells with immunological competence. This work has since been confirmed by Law et al[13].

At present, then, it would appear that the thymus normally carries out its immunological function both by producing cells which migrate to peripheral lymphoid tissue, and also by releasing a humoral factor. The relative importance of these two mechanisms remains, as yet, unknown.

The Neuromuscular Mechanism

In order to relate the thymus to myasthenia gravis, it is necessary to consider the normal physiology of the neuromuscular junction, the possbile mechanisms by which the normal can be interfered with, and the particular mechanisms of interference which have been implicated in myasthenia gravis.

1) *Normal Physiology*

Zacks, in a recent publication, has prepared a comprehensive review of the neuromuscular junction.[14] The transmitter substance acetylcholine is synthesized from choline and acetate ,in the presence of adenosine triphosphate, coenzyme A, and choline acetylase, in a site which is, as yet, not clear. The acetylcholine appears to be stored in the presynaptic membrane in what are known as synaptic vesicles. Even at rest, there is a continous release of a number of quanta or molecular packets of acetylcholine, resulting in the production of recordable miniature end plate potentials. These miniature potentials have been shown to be reduced by curare, a competitive acetylcholine blocker, and increased by neostigmine, an anticholinesterase agent.[15 16] Stimulation of the nerve leads to the formation of the nerve action potential which results in a breakdown of synaptic vesicles, releasing about one hundred times the resting number of quanta of acetycholine.[17] The acetylcholine is then thought to reach the motor end plate and combine with a chemoreceptor substance, probably located in the subneural apparatus. The complex formed appears to non-specifically increase the permeability of the post-synaptic membrane to ions available in the surrounding solution. The resulting ion flow generates the endplate action potential which, in turn, gives rise to the propagated muscle potential, and this is associated with mechanical shortening of the muscle fibre. The entire system is restored by the action of acetylcholinesterase, which hydrolyzes acetylcholine leaving the endplate again capable of being depolarized. This hydrolysis also produces choline and acetate for the resynthesis of the necessary acetylcholine.

2) *Possible Sites of Interference*

Theoretically, the primary defect in myasthenia gravis could be anywhere from the anterior horn cell to the muscle fibril itself. Bothelho, for example, found a discrepancy between the electrical activity of myasthenic muscle and its force of contraction,[18] suggesting a primary muscle fibre defect. However, the bulk of the evidence does indicate a primary defect in the acetylcholine mechanism of the terminal nerve arborizations and the motor endplate. This conclusion is based on the response to drugs known to be active at the neuromuscular junction,[19 20 21] and the electromyographic appearance of the myasthenic defect.[22] In addition, pathologically, it is the terminal nerve arborizations and the motor end plates which do seem primarily involved in myasthenia gravis.[14] However, even if one accepts the hypothesis of a disturbed acetylcholine mechanism, the specific defect involved remains unsettled. There may be:

I) Insufficient depolarization of the endplate:

A) Decreased synthesis or release of acetylcholine.

B) Competitive inhibition of acetycholine by an agent which does not produce depolarization.

C) The desensitizing type of block.[23] The depolarizing action of acetylcholine is inhibited, but unlike the competitive block, additional acetylcholine will not reverse it. (Presumably, the desensitizing block is the re-

sult of a change in the endplate receptor molecules from an effective to a desensitized or acetylcholine-insensitive state).

II) Persistent depolarization of the endplate:

A) Presence of a stable depolarizing agent.

B) Persistence of acetylcholine as a result of cholinesterase inhibition.

3) *The Defects Implicated in Myasthenia Gravis*

Two main schools of thought as to the specific site of the myasthenic defect have arisen, on the basis, chiefly, of the findings of light and electron microscopy.

A) Presynaptic Defect

The abnormalities in the terminal nerve arborizations have prompted Coërs and Desmedt,[24] and others, to conclude that the primary defect is presynaptic, and due to a deficiency in acetylcholine release, perhaps because of impaired acetylcholine synthesis. Riker has, in fact, shown that neostigmine and curare may exert their effects on the nerve terminals rather than the endplate.[25] In addition, Brody and Engel have recently demonstrated, in myasthenic muscles, groups of small muscle fibres indicative of denervation,[26] providing further evidence for a presynaptic lesion.

B) Postsynaptic Defect

Zacks, has pointed out that the changes seen in the terminal nerve arborizations are non-specific, and are seen in a number of myopathies.[14] However, the pathological changes in the endplate subneural apparatus do seem to occur only in myasthenia gravis. He, therefore, suggests that, in this disease the alterations in the terminal arborizations are secondary to a primary defect in the motor endplate, which appears to undergo a continuous cycle of degeneration followed by regeneration. The observed loss of material from the muscle membrane within the secondary synaptic clefts, the clefts between the radiating folds of the endplate subneural apparatus, can explain the functional deficit in one of two ways. There may be a decrease in the available postsynaptic area for acetylcholine binding; or, there may be an actual molecular abnormality in the post-synaptic membrane, specifically in the acetylcholine receptor substance. In either case, the end result would be a decrease in the amount of acetycholine acting at the endplate, leading to insufficient depolarization. The fact that not all myasthenic endplates obtained at biopsy show the characteristic changes can be explained by postulating that the exacerbations and remissions so characteristic of myasthenia, might be due to crises of widespread endplate destruction followed later by regeneration of the endplates.

This theory is compatible with the myasthenic crisis which would presumably result from the destruction of a sufficient number of endplates. The theory does not, however, offer a satisfactory explanation for the abnormal response, first observed in myasthenics by Churchill-Davidson and Richardson,[27] to decamethonium. This drug, a depolarizing type of blocking agent, combines with the acetylcholine receptor substance, and, initially, increases the amplitude of the action potential and the strength of myasthenic muscle. In a short time, however, both these parameters fall below what they were initially.

Myasthenia Gravis and the Thymus — Fact and Fancy

The hypothesis that the thymus plays a role in the etiology and pathogenesis of myasthenia gravis is, indeed, attractive. At present, however, such a statement is purely hypothesis. To date, no abnormality of thymic function has been definitely correlated with the presence of myasthenia. Certainly, pathological changes are usually observed, in the form of thymic hyperplasia or thymoma. Germinal centres containing proliferating lymphocytes and plasma cells may be seen in the thymic medulla, whereas normally they are not.[28 29] These changes, however, have no known associated abnormalities in thymic function. One can merely speculate as to their significance. Current thought views the pathology as evidence of an autoimmune process, and Burnet's work with New Zealand Black mice[28] would seem to support this concept.

Let us, for a moment, accept Burnet's view that the thymic follicles represent abnormal functioning "forbidden" clones which are ultimately disseminated to other lymphoid structures.[30] There are, still, a number of questions which must be answered. For example, what is the antigen with which these clones will react? Strauss and his co-workers have demonstrated the presence of globulin on alternate striations of myasthenic muscle fibres,[31] but this does not account for the neuromuscular defect seen in myasthenia. In addition, it does not agree with the evidence suggesting an abnormal neuromuscular mechanism as the primary myasthenic defect.[19 20 21 22]

Another major problem facing those who advocate Burnet's hypothesis, is the elucidation of the mechanism by which the "forbidden" clone is formed. Is there an antigenic change in the neuromuscular apparatus primarily? Or, is there normally a blood-thymus barrier which breaks down in myasthenia, allowing "self" antigen to enter the thymus and stimulate the production of forbidden antibody? Or, finally, is there a primary abnormality in the thymus with a *de novo* proliferation of the forbidden clone?

The work of Marshall and White[32 33] did seem to suggest that normally there was a blood-thymus barrier, and that only by direct injection of antigen into the normal thymus could specific antibody production be induced. However, a recent study by Sainte-Marie[34] contradicts this hypothesis. By injecting bovine serum albumin intramediastinally, he showed that antigen which drained into the mediastinal lymph nodes also penetrated the thymic parenchyma and entered thymo-

cyte cytoplasm. It si apparent, then, that the evidence for a blood-thymus barrier, is at best, doubtful.

There is another way in which the thymus could play a part in the etiology and pathogenesis of myasthenia gravis. Prior to the current view of myasthenia as an autoimmune disease, many investigators felt that the thymus was involved by virtue of the release of neuromuscular blocking agents. In fact, Wilson et al[35 36] claimed to have isolated, from thymus, quaternary nitrogen compounds. Recently, they have demonstrated quantitative and qualitative differences in these compounds in myasthenic as compared to normal thymus.[37] In addition, there have been numerous reports suggesting the presence of circulating neuromuscular blocking agents in myasthenia gravis.[38 39] However, there are other studies which contradict these findings,[40 41] and it should be noted that even if such agents do exist, it would be difficult to explain how they could account for the pathological changes seen in the neuromuscular junction.

The suggested relationships between myasthenia gravis and the thymus are based on some facts and much fancy. We have included the fancy in these reviews, in the hope that these are the hypotheses from which the facts of the future will emerge.

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REFERENCES

1. Weigert, C.; Neurol. Centralb. 20: 597, 1901.
2. Miller, J. F. A. P.; Ann. Inst. Pasteur 105: 1007, 1963.
3. Miller, J. F. A. P.; Lancet 2: 748, 1961.
4. Archer, O. K., Pierce, J. C., Papermaster, B. W., and Good, R. A.; Nature 195: 191, 1962.
5. Good, R. A., Dalmassa, A. P., Martinez, C., Archer, O.K., Pierce, J. C., and Papermaster, B. W.; J. Exp. Med. 116: 773, 1962.
6. Globerson, A., Fiore-Donati, L., and Feldman, M.; Exp. Cell Res. 28: 455, 1962.
7. Globerson, A., and Feldman, M.; Transplantation 2: 212, 1964.
8. Miller, J. F. A. P.; Nature 195: 1318, 1962.
9. Cross, A. M., Leuchars, E., and Miller, J.F.A.P.; J. Exp. Med. 119: 837, 1964.
10. Burnet, F. M.; Australian Journal of Medicine 2: 7 1962.
11. Yunis, E. J., Hilgard, H., Sjodin, K., Martinez, C., ar Good, R. A.; Nature 201: 784, 1964.
12. Osoba, D., and Miller, J. F. A. P.; J. Exp. Med. 11 177, 1964.
13. Law, L. W., Trainin, N., Levey, R. H., and Bart W. F.; Science 143: 1049, 1964.
14. Zacks, S. I.; The Motor Endplate, W. B. Saunde Company, London-Philadelphia, 1964.
15. Fatt, P., and Katz, B.; J. Physiol. 115: 320, 1951.
16. Fatt, P., and Katz, B.; J. Physiol. 117: 109, 1952.
17. Schwab, R. S.; Int. J. Neurol. 2: 224, 1961.
18. Bothelho, S. Y.; J. Clin. Invest. 34: 1403, 1955.
19. Walker, M. B.; Lancet 1: 1200, 1934.
20. Bennett, A. E., and Cash, P. J.; Arch. Neurol. ar Psychiat. 49: 537, 1943.
21. Grob, D., Johns, R. J., and Harvey, A. M.; Joh Hopk. Hosp. Bull. 99: 219, 1956.
22. Lindsley, D. B.; Brain 58: 470, 1935.
23. Katz, B., and Thesleff, S.; J. Physiol. 138: 63, 1957
24. Coers, C., and Desmedt, J. E.; Lancet 2: 1124, 195
25. Riker, W. F.; A.M.A. Arch. Neurol. 3: 488, 1960.
26. Brody, I. A., and Engel, W. K.; A.M.A. Arch. Neur 11: 350, 1964.
27. Churchill-Davidson, H. C., and Richardson, A. T.; Neurol. Neurosurg. Psychiat. 15: 129, 1952.
28. Burnet, F. M.; Scientific American; 207: 50, 1962.
29. Viets, H. R., and Schwab, R. S.; Thymectomy for M asthenia Gravis. Springfield, Illinois. Thomas, 1960.
30. Mackay, I. R., and Burnet, F. M.; Autoimmune D seases. Springfield, Illinois. Thomas, 1963.
31. Strauss, A. J. L., Seegal, B. C., Hsu, K. C., Burkholde P. M., Nastuk, W. L., and Osserman, K. E.; Proc. So Exp. Biol. Med. 105: 134, 1960.
32. Marshall, A. H. E., and White, R. G.; Brit. J. Ex Path. 42: 379, 1961.
33. Marshall, A. H. E., and White, R. G.; Lancet 1: 103 1961.
34. Sainte-Marie, G.; J. Immun. 91: 840, 1963.
35. Wilson, A., Obrist, A. R., and Wilson, H.; Lancet 368, 1953.
36. Nowell, P. T., and Wilson, A.; Myasthenia Grav The Second International Symposium Proceedin Edited by Henry R. Viets, M. D. Springfield, Illino Thomas, 1960. pp238-256.
37. Calvey, T. N., and Wilson, A.; Brit. Med. J. 5355: 46 1963.
38. Walker, M. B.; Proc. Roy. Soc. Med. 31: 722, 1938
39. Wilson, A., and Stoner, H. B.; Quart. J. Med. 13: 1944.
40. Schwarz, H.; Canad. M. A. J. 67: 238, 1952.
41. Lammers, W., and Most Van Spijk, D.; Nature 17 1192, 1954.

Tumour Antigens

K. Pritzker 6T7

Early hopes that neoplastic cells were in some fashion antigenically different from normal cells, and that an anti-tumour vaccine might be produced, sparked the entry of immunochemistry to the field of cancer research. A simple anti-serum, containing antibodies produced by injecting cancer cells into an allogenic† or xenogenic‡ animal cannot be effective, for the vast majority of antigens in a tumour cell are identical to those of the normal cell. Consequently, almost all antibodies in this anti-serum would attack the host as well. Thus, a search began for specific antigens in tumour cells for which antibodies could be developed. The immunologist's elegant techniques and his strange, at times, bewildering jargon, reflect the difficulties and the sophistication of this search. The immunological activity of antigens, discovered or conjectured, is the subject of this article.

A specific antigen of a neoplastic cell is a molecule produced or possessed by that cell and not possessed by the host cells. This molecule can elicit an immune response, either in the host or in some other medium. Since the response varies with the nature of the antigen, and the method of detection, the response in each case presented will be discussed. Furthermore, antigens discovered by *in vitro* techniques do not necessarily have antigenic activity *in vivo*, for reasons to be considered later.

Before discussing the role of tumour antigens, the improbability that the antigens have no role should be mentioned. Cells with antigenic distinction are likely to be selected against by host mechanisms, preserving the integrity of "self". Therefore, implicit with the presence of tumour antigens is a characteristic either of the antigens or of the host which ensures the survival and growth of the cancer cell in spite of the host's defence mechanisms.[1]

Now, for the antigen to induce a response, the host tissues must recognize the antigen. This infers that the host must have a "sensor" mechanism which, activated in the presence of antigen, produces the immune response. The recognizability of antigen structure, the limitations of the "sensor", and the organism's general capacity for immune response, are closely interrelated factors. These factors, of course, vary with the individual and with the antigen. Therefore, tumour antigens induce varying degrees of response in the host. Rarely, however, is circulating antibody found.

It is speculated that spontaneous neoplastic changes in cells might be quite common. Most of these changes are sufficiently antigenic to provoke an immune response that will destroy these aberrant cells. Only rarely, when the antigens are not recognizable, or when the capacity for response is decreased, is carcinogenesis thought to occur.[1,2] The various factors, antigen, "sensor", and capacity for immune response, might interact to promote carcinogenesis *in vivo*, in one or more of the following ways.

1) Since the term "tumour antigen" is used to include antigens found in subcellular fractions, the antigen may be intracellular, and thus inaccessible to the host's defence mechanisms. Or, the antigen may not be accessible because it is combined with another substance *in vivo*. For the host to recognize an antigen then, it must be available in, or on the cell membrane.[2] Failure to recognize the antigen, of course, will allow the cancer cells to grow and multiply.

2) The antigen may not exist in sufficient quantities for the host to recognize, at first. By the time the antigen concentration becomes sufficient for the "sensor" to detect, the malignant cells would be proliferating too rapidly. If the host's response were inadequate, malignancy would ensue. Again, inadequacy of response might be due to inaccessibility of the antigens on cells in the middle of the rapidly growing tumour. Because of the difficulty of examining a few cancer cells present in many thousands of cells, model experiments have been done. Boyse,[1] transplanting tumour cells (induced chemically with methylcholanthrene) to isogenic* strains of mice, found that an inoculum of as few as 40 cells would produce a tumour, whereas an inoculum of about 12,500 cells would sometimes regress. Humphreys et al[1], have reported that the use of unusually small inoculi may increase the rate of growth of transplanted leukemias in isogenic mice. The large inoculum, with much more antigen, produced a quicker host response, which was adequate to destroy the tumour. The small inoculum, however, produced less host response. This allowed it to remain in the host and start to grow. This work also illustrates how a few remaining cells of a treated tumour might give rise easily to a new tumour.

3) The antigen molecule may not differ in structure significantly from the corresponding host molecule to be recognized as "non-self". The difference might well

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†allogenic (allo- another, + -genic) = homologous ≡ animals of the same species who are genetically different, i.e. Not inbred strains or uniovular twins.

‡xenogenic (xeno- foreign, + -genic) = heterologous ≡ individuals of different species, e.g. human and rabbit. These terms, coined for transplantation biology, are now in standard use.

*isogenic (iso- equal, + -genic produced or born) ≡ animals which contain identical genetic complement, e.g., uniovular twins, inbred strains of mice. (New England J. of Medicine 271: 502, 1964.)

be of the order of a few amino acids. If the tumour antigen could be rendered recognizable, then the host might produce specific antibody. Interesting experiments along this line have been done by Cinader.[3] At birth, allogenic rabbits were injected with bovine albumin. These animals, then, were tolerant, i.e. they did not form antibodies against bovine albumin, when it was injected in later life. An azo-group was attached to alter the bovine albumin slightly. This was then injected into the tolerant rabbits. In 80-90% of the animals, no antibody response was found. But, when human albumin was injected into rabbits tolerant to bovine albumin, antibodies formed against the human albumin. Remarkably enough, after a while, these antibodies broadened their specificity and removed bovine albumin from plasma. A similar phenomenon was observed in the 10% of animals which produced anti-bodies to azo-albumin. In theory, if we could isolate a tumour antigen, and if we could alter it chemically so that we could induce an antibody response specific for the altered antigen and the original antigen (but not the host molecule), we might destroy the tumour by administration of the altered antigen.

In practice, seemingly insurmountable difficulties reduce the promise of this approach. In all tumours, many non-specific antigens give positive serological reactions. These include dying and necrotic cell fragments, Forssman antigens, passenger viruses, and degenerated blood and connective tissue elements.[2] To isolate a molecule that differs minutely from the normal molecule, that exists in a tiny concentration compared to the total tumour cell, and that may be confused with non-specific antigenic substances is a formidable difficulty indeed! To alter the antigen so that the antibody will broaden its specificity to include the original antigen, and so that this response will occur in all individuals (recalling that only 10-20% of azo-albumin injected rabbits produced antibodies), is still another problem. Moreover, although some tumours have antigens in common with other tumours but not with non-tumourous tissue, no single antigen has been found, as yet, common to all carcinomas of man.[2] With carcinomas induced chemically in isogenic rats, even though two tumours were induced by the same carcinogen at the same site, even though these were indistinguishable microscopically, they had antigens quite distinct from one another.[1] Therefore, these antigens appear not to have a direct relationship to the molecular configuration of the carcinogen. This implies that a suitable antigen would have to be isolated from each individual chemically induced tumour, a task far beyond our present analytical abilities. This dreary, depressing situation has been ameliorated, somewhat, by virus research. Different tumours, experimentally induced by the same virus, possess a common antigen, long after the virus or the viral antigen can be demonstrated in the tissue.[7]

4) The antigen may be too small to evoke an adequate immune response. Such a substance is malignolipin, a simple phospholipid, found in some human tumours. This substance may act as a weak hapten. However, its accessibility remains questionable.[2]

5) Instead of failure to recognize the antigen, the antigen might elicit a response which contributes to the malignancy of the tumour. As some experimental tumours develop, there is an increase in cephalin/lecithin lipoprotein ratio found in the cell membrane. Green has suggested that the carcinogen becomes bound to the lecithin lipoprotein rendering it antigenic. Tumour cells, he suggests, are produced with varying amounts of cephalin/lecithin. But, as the cells with high lecithin concentration tend to be destroyed by combination with antibodies, the cells which survive and proliferate are progressively richer in cephalin. The selection process would continue until the nature of the cell membrane had changed considerably, since the cephalin and lecithin lipoproteins have different charges. Increased cephalin lipoprotein increases the negative charge, leading to decreased adhesiveness and contact inhibition between cells, thereby facilitating invasion and metastasis. (The role of calcium ion deficiency in the same phenomenon was not discussed in this model.) With these tumours, lecithin or ethanolamine retard carcinogenesis. Presumably, these compounds bind the antibody and thus prevent the selection of metastatically capable cells. Based on this and other evidence Green[4,5] has extended his ideas to an immunological theory of carcinogenesis.

This theory distinguishes two immune responses which would combine to promote carcinogenesis. First, hypothetical tissue specific antigens called "self-markers" exist on all normal cells. An immunological process, hypothetically a part of the host's growth regulatory system, would recognize the "self-marker" and prevent invasion and proliferation of the cells beyond normal tissue boundaries. Essentially, the immune response would produce a substance (antibody or hormone) which could bind itself to the "self-marker". By this means, the substance could either destroy the cells of the tissue periphery, or prevent these cells from reproducing. In the latter case, it is presumed that the "self-marker" is also involved in the replicating mechanism of the cell. In either case, since all cells of a tissue have "self-marker", the theory does not explain why the cells on the periphery of a tissue are inhibited whereas the other cells are not. Overlooking this difficulty it is next suggested that carcinogens would combine with the "self-marker" forming a complex which is different antigenically. Consequently, control of growth by the above mechanism is lessened. The complex then elicits a second antibody response which tends to destroy the carcinogen- "self-marker" complex cells. Because carcinogenesis occurs a considerable time (several cell generations) after introduction of the carcinogen, it is assumed that the carcinogen- "self-marker" complex is replicated in daughter cells. But, in a few daughter cells, the complex is not transferred from the parent

Becase they lack the complex, the second antibody response does not destroy these deprived cells; because they lack the "self-marker", these cels are not recognized by the host's first immune response. Therefore, the cells become capable of malignancy. The complex might be deleted spontaneously (somatic mutation), possibly as a result of antibody combining with the complex in the parent cell. Actual evidence for the "self-markers" and the two immune responses described is slim. This hypothesis illustrates, however, our need to know more about the regulation of normal cell growth and the place of the immune response in this. Further, it may be seen from the above discussion, how an immune response may be present, yet the tumour may grow independent of it. Also, this theory puts forth an explanation for antigen deletion, a phenomenon described below.

6) In a few reported human cases, and in some experimental work, tumours have spontaneously regressed. In some patients whose primary lesions were destroyed, complement-fixating antibodies have been demonstrated. Southam et al. found that human cancers implanted in healthy volunteers were rejected. (allograft reaction). But, tumours implanted in cancer patients continued to grow until they were excised.[6] Occasionally, patients, with previously regressed or "cured" cancers, after a long latent period, have been exposed (for other reasons) to ionizing radiation sufficient to dimminish immunological responses.[7] Unfortunately for these patients, recurrence of their tumours occurred shortly after exposure. Moreover, with many malignancies, a "reactive hyperplasia" of lymphoid tissue has been observed.[7] Common to the explanation of the disconnected phenomena observed above could be ill understood general immunological mechanisms which retard malignancy. In cancer patients these mechanisms are, presumably, suppressed.

The subject of tumour antigens now includes antigens present in the host, but not present in the tumour. For example, with rat hepatomas, induced with 4-dimethylaminoazobenzene (DAB) type compounds, antigens were found in subcellular fractions of livers in normal animals, but not in hepatomas or liver of susceptible isogenic animals.[5] Essentially, this antigen loss is a protein loss. Whether this loss has an immunological basis as suggested by Green, or whether this loss represents a biochemical combination of a carcinogen complex, or whether this loss reflects the undifferentiation of the cancer cell, thereby only incidentally related to carcinogenesis, is not known.

Work on tumour antigens, present or deleted, has led to the study of these antigens for themselves as biochemical entities. The weak or unrecognizable antigen may be a viral product, or a chemical carcinogen complex, or a spontaneous, or ionizing radiation induced intracellular mutation. The biochemical activity of many of these antigens may be quite apart from their antigenic activity. Any discussion of biochemical activities of these antigens would involve detailed consideration of proposed carcinogenic mechanisms. Omitting the discussion here, then, is not unpardonable.

CONCLUSIONS

No agreement exists on the immune response in cancer. On one hand, the weak antigen, because it is masked intracellularly, or because of its structure, or because it exists in insufficient concentration, or because the responsive system is depressed, fails to elicit a satisfactory immune response. On the other hand, the immune response, itself, might promote carcinogenesis. The broad definition of tumour antigen, the lack of immune response *in vivo* in some cases, and the hypothetical presence of tumour antibodies in others, have added to the confusion in this field. Certainly, the work on tumour antigens has contributed a new and powerful method for the biochemical investigation of the cancer cell. Both the variety of speculation surrounding the available data and the confounding variety of the data found, indicate that work on tumour antigens is only just beginning.

REFERENCES

1. Boyse, E. A. Immune Response to Experimental Tumours. Guys' Hospital Reports 112: 433–448, 1963.

2. Busch, H. Introduction to the Biochemistry of the Cancer Cell. pp. 202 et seq. Academic Press, New York, 1962.

3. Cinader, B. Acquired Tolerance, Autoantibodies, and Cancer. C.M.A.J. 86: 1161–1163, 1962.

4. Green, H. N. Immunological Aspects of Cancer. Ciba Foundation Symposium, "Carcinogenesis, Mechanisms of Action". J & A Churchill Ltd., London, 1959.

5. Heiger, I. Carcinogenesis. pp. 31–43, Academic Press, New York, 1961.

6. Boyd, W. Textbook of Pathology, pp. 197–198, Lea & Febiger, Philadelphia, 1961.

7. Woodruff, M. F. A. Immunological Aspects of Cancer. Lancet, 2: 265–270, August, 1964.

8. Russel, P. S. and Moraco, A. P. Biology of Tissue Transplantation. New Eng. J. Med. 271: 502, 1964.

Abstracts

Surgical Treatment of Transposed Great Vessels

Trusler, G. A., Mustard, W. T., Fowler, R. S.: Can. Med. J. 91: 1096-1100, 1964.

Early total correction of transposed great vessels is advocated to avoid subsequent, inevitable pulmonary vascular disease. The authors report on the rationale and results of creation of atrial septal defects in children with complete transposition of the origins of the aorta and pulmonary artery, one of the commonest and most serious congenital cardiac lesions.

An atrial septal defect in these cases unites two independent and inefficient circulatory systems — the pulmonary and systemic circuits. This procedure results in unequivocal improvement of the patient's condition but it does not effect a long-term cure. Emphasis is, therefore, placed on regarding this as the first stage of a two-stage procedure in attempt to achieve total correction of the transposition. Further improvement of the technique for complete correction is essential.

M.T.

Insulin and Glucose Transport

Henderson, M. J.: Can. J. Biochem. 42: 933-944, 1964.

The perfused rat heart was used to determine the role of insulin in glucose transport in muscle cells. The several possible means for glucose transfer into cells have been discussed viz. diffusion, pores, pinocytosis, carriers and dimerization; and arguments in favor of the carrier theory have been elaborated. Glucose uptake was considered to consist of three steps: (1) passage from the capillary to the cell surface, (2) transport across the cell membrane and (3) metabolism within the cell. The first takes places by diffusion, is not limiting under normal circumstances and is not influenced by insulin. Transport across the cell membrane is mainly under insulin control and is the major rate-limiting step in glucose uptake when extracellular glucose levels are in the normal range. Metabolism of glucose within the cell is the major rate-limiting step in glucose uptake when intracellular concentration is so high that its phosphorylation is near saturation.

M.T.

Hepatic Veography

Rappaport, A. M. et al.: Gastroenterology 46: 115-12 1964.

Hepatic venography is presented as an important ne diagnostic technique. Hepatic venograms were obtaine in dogs by complete occlusion of the intrahepatic po tion of the inferior vena cava by a specially devise catheter inserted via the femoral vein and by injectio of radio-opaque dye through this catheter. Cine film and spot films were taken. The entire venous outflo is temporarily blocked and venous radicles are di tended so that much greater structural detail resul than from arteriograms or portograms.

A similar technique was used in a few patients fo hepatic and renal venograms with no side effects an excellent results. The authors showed characteristi radiographic deviations from normal in cirrhosis an liver metastases to illustrate the usefulness of this impo tant new technique in diagnosis of these and other d seases.

M.T

Bladder Control in Female Paraplegics

Jousse, A. T., Macdonald, M., Wynne-Jones, M.: Para plegia 2: 146-152, 1964.

A program of training for female paraplegics is ou lined which permits mastery of useful bladder contr for patients with complete or partial transection of th spinal cord or cauda equina. Best results are obtaine with cooperative, well-motivated patients who unde stand the purpose of the program and who have rea sonable agility in shifting themselves about. The sam approach is used for patients with complete or parti lesions, spastic or flaccid bladders.

Persistance for weeks or months using various ap proaches may be necessary but an unsuccessful attemp should not be prolonged beyond 2-4 hours since it onl leads to fatigue, discouragement and even sepsis.

Greater success is achieved with patients with parti lesions since they may have a degree of residual volun tary control while the presence of some sensory aware ness results in better control of incontinence and earlie detection of complicating infections.

M.T

Infectious Mononucleosis Complicated by an Acute Haemolytic Process

E. A. T. Salmon 6T6

Infectious mononucleosis is an acute infectious disease of unknown etiology. It has a self-limited course, and is characterised by lymphocytosis with atypical lymphocytes in the peripheral blood.

The absence of anaemia in infectious mononucleosis is a generally accepted finding, and, indeed, is useful as an aid in differentiating the disease from leukemia. Bernstein[1] has stated that anaemia of any appreciable degree does not appear in infectious mononucleosis, unless associated with some dietary deficiency or haemorrhagic disorder. However, many cases have been reported in which a certain degree of anaemia is present, and is not associated with either of the above. The occurrence of infectious mononucleosis complicated by a haemolytic process has been reported, but so far there has been no report of any causative antibody.[2] It is the purpose of this paper to describe such a case and the antibody detected.

CASE HISTORY

A woman, aged 22, was admitted with the clinical diagnosis of infectious mononucleosis. A haemolytic anaemia developed and was treated with cortisone.

Laboratory Findings

Haemoglobin	8.0 gms.
W.B.C.	4,000/cu mm.
E.S.R.	23 mm/hr.
Differential	*Polymorphonucleocytes*-1,240
	Lymphocytes-2,480
	Monocytes-280

Smear: Atypical lymphocytes present.

MATERIAL, METHODS AND RESULTS

A screening Paul-Bunnell test was performed, using unheated serum (patient's) against a 10% suspension of washed sheep cells. A positive test was obtained, as indicated by agglutination within one minute.

A portion of the remaining serum was inactivated at 56°C. for half-an-hour, and examined by Davidsohn's adsorption technique.[3] In this technique the inactivated serum is diluted 1:5 in each of guinea pig kidney suspension and ox red cell suspension, and incubated overnight at 4°C. The guinea pig kidney suspension absorbs the Forssman, or species, antibody, while the ox cell suspension absorbs the heterophile, or glandular fever, antibody. For the actual titration, three rows of ten tubes are required:

1. Unabsorbed serum, serially diluted from 1/5 to 1/2560 with saline;
2. Guinea pig-absorbed serum, serially diluted 1/5 to 1/2560 with saline;
3. Ox cell-absorbed serum, serially diluted 1/5 to 2560 with saline.

To each tube an equal volume of 0.40% sheep red blood cell suspension is added, shaken, and the rack of tubes is incubated at 37°C for 2 hours. A positive result (i.e. red cell agglutination at titre above 1/80) in the unabsorbed and guinea pig-absorbed sera indicates the presence of a heterophile antibody; on the other hand, if the antibody is completely absorbed by both the guinea pig kidney and ox cell suspensions then the patient is probably suffering from serum sickness.

On absorption of this patient's serum, it was found that the ox cells did not absorb the heterophile antibody, shown previously to be present by the screening Paul-Bunnell, as it should, and the test was repeated using different batches of ox-cells. The results however, were the same each time, as seen in Table 1. Therefore, this suggested the presence of an interfering antibody.

Table 1: Results of Davidsohn's absorption technique.

	1/5	1/10	1/20	1/40	1/80	1/160	1/320	1/640	1/1280	1/2560	1/5120	1/10240	1/20480
Unabsorbed inactivated serum	5+	5+	5+	4+	4+	3+	3+	3+	2+	2+	2+	+	W
Guinea pig absorbed serum	5+	5+	5+	4+	4+	3+	3+	2+	2+	+	W	—	—
Ox cell absorbed serum	5+	5+	4+	3+	2+	2+	+	W	—	—	—	—	—

The results of the agglutination tests are shown.
Degree of agglutination is graded 5+ to weak (w); — = No Agglutination.

Table II: Results of enzyme-modified indirect Coomb's test.

GROUP O CELLS	R_1*	R_2	R_1R_2	rr	rrK+	R′	R″	Patient's own cells
unabsorbed Patient's serum	(+)	(+)	(+)	(+)	(+)	w	w	(+)
absorbed Patient's serum	—	—	—	—	—	—	—	—

Table II shows that the antibody was completely absorbed by the patient's own papain-modified cells; hence, there was n "hidden" antibody present, but in fact, an auto-antibody.

When the freshly collected blood was shown to contain cold agglutinins, it was thought that these were the antibodies interfering with the reaction of the ox cells. To rule out the possibility of other common agglutinins, the patient was grouped (Group A positive), and antibody tests performed. Testing of the patient's serum in albumin was negative, as was the indirect Coombs test.[3] However, agglutination was obtained with all enzyme (papain)-treated cells, including the patient's own cells, weakly at 37°C, stronger at 18 - 20°C, and very strongly, at 4°C, again indicating the presence of a cold agglutinin. From previous experience it was known that enzyme-modified cells are sometimes more sensitive than the indirect Coomb's test for the detection of weak antibodies. In order to exclude this possibility, the patient's washed packed cells were enzyme-modified with papain (ficin is a better proteolytic enzyme but is more corrosive), and these washed modified cells were then used to absorb this papain-active constituent from the patient's serum. After absorption at 37°C for ½ hour the sample was spun, and the serum was removed; aliquots were added to a panel of cells containing as many antigens as possible, using the papain technique. At the same time, the unabsorbed serum was put up against the same panel of cells using the same techniqu The papain technique was carried out in the followir order:

1 vol. of the patient's serum (unabsorbed or absorbed was put in a tube initially. To this, 1 vol. of papai and then 1 vol. of 5% washed Group O cell suspensio were added. Incubation at 37°C was immediately carrie out, without mixing, for 1 hour; microscopic examin tion was then performed. The results obtained are show in Table II.

Further evidence for the presence of a cold agglutini was provided by negative Donath-Lansteiner, Ham, an paroxysmal cold hemoglobinuria tests. The results of th direct Coombs test also suggested the presence of cold antibody (Table III).

Having demonstrated the presence of cold auto-ant bodies, and realising that these are often directed agains the *I* antigen,[6 7 8 9] an effort was made to determin whether this patient's serum contained antibodies of specificity. The patient's serum was, therefore, put u against: (1) a number of papainized adult group O cel which contained *I* antigen, and (2) cord blood group cells which contained *i* antigen. These were incubated a

Table III: Quantitative Antiglobulin Test

Dilution of Coombs Reagent	1/4	1/16	1/64	1/256	1/1000	1/4000	1/16,000
Patient's washed cells	3+	2+	2+	W	—	—	—
ORh+ cells sensitized with weak Rh Antibodies	2+	2+	4+	5+	5+	5+	3+
Group O Rh- sensitized cells (Control)	2+	—	—	—	—	—	—
Group AB Rh- cells (control)	2+	—	—	—	—	—	—

These results, using the patient's own cells, are characteristic of a cold antibody. The typical result in the presence of a warm antibody is shown by the Group O Rh+ sensitized cells. The degree of agglutination is indicated as in Table I.

* **Fishers nomenclature of the Rh blood groups.**[3]

R_1 = CDe
R_2 = CDE
R_1R_2 = CDe/CDE
rr = cde
K+ = Kellog positive
R′ = Cde
R″ = CdE

4°C for 2 hours. Bombay blood which contained *I* antigen was used for control as were adult *I* negative cells. All the cells used contained the *H* antigen, except the Bombay blood. The results obtaned are indicated in Table IV.

Table IV shows that:

(1) the patient's serum reacts against cells containing *I* antigen;

(2) the reaction is directed against *I*, and not *H* antigen, as shown by the reaction against Bombay Blood which contains *I* antigen, but no *H* antigen;

(3) the patient's own cells contain *I* antigen;

(4) an auto-immune reaction against *I* antigen was present in this patient.

DISCUSSION

Anti-*I* was the name given by Weiner *et al.* in 1956, to the antibody in the serum of a patient (Group A,B) with acquired haemolytic anaemia of the cold auto-antibody type.[11] Using the serum of this A,B patient to screen donors in New York, Weiner found only five people to be *I* negative, and these he called *i* positive. He assumed that these *i* positive people were homozygous *ii*, although there was no family study to verify this.

Further work on this subject has shown that there are differences in the strength of the *I* antigen.[7 8 9] One can, therefore, assume that the *I* system is analogous to the P system in that one can create sub-groups I_1, I_2, I_3, etc., or *I* strong, *I* weak and *I* negative (*i*). The similarity to the P system has been substantiated by the fact that hydatid cyst fluid, which is known to be an inhibitor of anti-P,[6 8] also inhibits anti-*I*, although a greater concentration is required in the case of anti-*I*. To complicate matters even more, variations in patients with *i* have been observed. Jenkins and Marsh[16] studied the case of a donor who was *I* negative, and had anti-*I* in his serum. He appeared to be lower down the *i* scale than the five people mentioned in Weiner's study. There was also a strong suggestion that there is some relationship between the *I* system and the ABO, in that the *I* antibody reacts more strongly to *I* antigen on O cells than to *I* antigen on cells of other ABO groupings.[8] In other patients, it was however, observed that some A_1 cells were more strongly agglutinated by anti-*I* than other groups, though A_1i cells gave no agglutination.[7] This reaction with A_1 cells is probably due to the fact that anti-*I* is sometimes accompanied by a "pseudo anti-A".

Three sources of anti-*I* have been recognised[6]:

(1) Auto anti-*I* from *I* positive people suffering from haemolytic anaemia of the cold auto-antibody type;

(2) Anti-*I* from people who are *I* negative;

(3) The weaker anti-*I* from *I* intermediate people not suffering from acquired haemolytic anaemia.

In these people, the cold autoantibody was previously labelled non-specific.

The *I* antigen is a *public* one; thus, the majority of the population would have this antigen. The titration of cold agglutinins with O cells at low temperature actually determines the strength of the *I* antibody present, better results being obtained with enzyme-modified cells.

The reaction observed in Table IV, with cord blood, is one of the characteristics of the I antigen. At birth, there is more *i* than *I*, but normally by the time adult life is reached, the *i* disappears with a concomitant increase in strength of the *I* antigen.[7 8] This is analogous to haemoglobin F in the newborn, which decreases and almost disappears, with the appearance of Haemoglobin A, in the normal adult.

The haemolytic crisis which develops as a result of glandular fever is only temporary, with the titre of the *I* antibody (previously called cold agglutinin or non-

Table IV

Patient's serum	1/2	1/4	1/8	1/16	1/32	1/64	1/128	1/256	1/512	1/1024
Adult Cells O*I*	V	V	V	3+	3+	2+	+	(+)	W	—
Adult Cells O*I*	V	V	V	3+	3+	2+	+	W	—	—
Adult Cells O*I*	2+	2+	2+	+	+	(+)	W	W	—	—
Cord Blood O*i*	(+)	W	—	—	—	—	—	—	—	—
Cord Blood O*i*	W	—	—	—	—	—	—	—	—	—
Adult O*i*	—	—	—	—	—	—	—	—	—	—
Patient's own cells	V	V	V	3+	3+	2+	2+	2+	2+	W
Bombay Blood O*I* (control)	V	V	V	3+	3+	3+	2+	2+	2+	+

The reaction is graded from v = complete agglutination to w = weak.

specific antibody) falling and the direct Coombs becoming finally negative, in the space of a few weeks. The case presented in this paper was clinically, haematologically, and serologically shown to be glandular fever. Although it was not noticeable at the onset of the disease, a mild haemolytic process was present, with the serum bilirubin elevated and the smear showing some hypochromia with reticulocytosis.

Jaundice is not uncommon in infectious mononucleosis uncomplicated by a hemolytic process and increasing evidence supports the view that impaired liver function accompanies the majority of cases of this disease.[14] The S.G.O.T. and S.G.P.T. levels are usually elevated. Jaundice may occur as the initial symptom, or later in the disease, but more frequently, hepatitis in the absence of jaundice has been reported. The usual duration of the hepatitis is 2 to 6 weeks, with a rapid return to normal.

Similarity of the Haemolytic Anaemia of Infectious Mononucleosis to that Caused by Known Viruses

Some patients with viral pneumonia may develop an acute acquired haemolytic anaemia. The causative antibody has now been shown to be *I* specific.[9] As with infectious mononucleosis, this is a temporary haemolytic process.

It seems possible that the virus of infectious mononucleosis, if it exists, has the property of stimulating the production of cold antibodies. However, it would appear that the titre must be above 1/256 for haemolytic anaemia to occur, and in most cases it is below this; hence, the incidence of an associated haemolytic episode is not high.

Auto-Immune Antibodies

As early as 1935 Belk suggested that some cold agglutinins may be active in infectious mononucleosis.[2] In studying a case of convalescent infectious mononucleosis he found in the serum, not only heterophile antibody agglutinins and haemolysins against sheep, horse, rabbit, and pig cells, but also auto-agglutinins which were active below 10°C but not at 37°C. This led him to the conclusion that a *non-specific* stimulus in this disease might result in widespread production of antibodies.

It is known that the body in health avoids forming antibodies against its own tissues, a tolerance developed in early foetal life. A breakdown of this tolerance to self results in the auto-immune state. In this patient such a situation is manifested by an auto-immune haemolytic anaemia. The cause of such an abnormal state is not clearly understood, but it was shown in this case that auto-antibodies were present which could be detected both on the patient's red cells and in her serum. In other cases the auto-immune antibody has been directed against a well known system such as Rh, anti-e being the first of the well known blood group antibodies to be detected as an auto-immune antibody.[4 12]

In most cases, if not all, of the auto-immune antibody type of acquired haemolytic anaemia, part, at least, of the auto-antibody is *pan-reacting* so that the patient's serum is incompatible, not only with his own red cells, but with those of almost all donors who could be chosen for him, if a transfusion were required. O negative blood is, however, sometimes compatible, anti-*I* apparently being the pan-reacting antibody.

The Role of Hypersplenism

Some authors[13] are of the opinion that hypersplenism can cause selective action on bone marrow and peripheral blood constiuents, resulting in haemolytic anaemia, thrombocytopenia, leukopenia, or some such combination, and that such a mechanism may act in infectious mononucleosis.

Splenomegaly is well recognized in infectious mononucleosis and occasionally, thrombocytopenia occurs but, haemolytic anaemia resulting primarily from hypersplenism seems unlikely.

Wright *et al*[13] suggested that the spleen may be involved in the production of incomplete antibodies, due to the fact that in some cases, such antibody could only be demonstrated by using splenic residual blood. This may be accounted for, if we take into consideration that the spleen is the important organ in which sensitized red cells are filtered from the circulation, trapped and sequestered, phagocytosed and lysed.

Lymphocytes and Antibody Production

It has been shown[4] that cells in the lymph nodes can produce antibodies, since more antibodies are contained in the fluid leaving lymph nodes than that entering it.

Roberts[4] has shown that antibody production appears to be initiated by the lymphocytes which, during the synthesis of the antibody, metamorphose to transitional cells, then to preplasma cells, and finally to plasma cells.

In infectious mononucleosis the predominant cell are the atypical lymphocytes, or virocytes, as they are sometimes called. Atypical lymphocytes are also found in such conditions as acute viral hepatitis, viral pneumonia, mumps, and German measles. In most of these there is some sort of antibody synthesis;[4] some of these may be cell-specific, as against red cells, resulting in auto-immune haemolytic anaemia. At times, other manifestations are seen, including thrombocytopenic purpura of the idiopathic type. In the benign lymphoproliferative disorder of infectious mononucleosis, one finds the heterophile antibody against sheep red blood cells and, more rarely, the "cold" auto-antibody — anti-*I* — directed against the patient's own cells and resulting in auto-immune haemolytic anaemia.

SUMMARY

Anti-*I*, a cold auto-antibody, has been isolated in a case of infectious mononucleosis complicated by an auto-immune haemolytic process. Being a cold complete antibody, it naturally falls into the 19S globulin fraction, and is, thus, one of the macro-molecular antibodies. It may, therefore, be assumed that this antibody, like all similar antibodies, would not cause haemolytic disease of the newborn if it were produced during pregnancy.

Patients with an anti-*I* haemolytic anemia of severity sufficient to warrant blood transfusion should be given *I* negative blood.

Methods for the detection and identification of the *I* antibody are cited. It would seem useful to perform a direct Coombs test on all cases of infectious mononucleosis with low haemoglobin. A cold agglutination test should also be done, preferably using enzyme-modified cells (papain or ficin).

ACKNOWLEDGEMENT

This paper could never have been produced without the help and criticism of several people, and for this, I am most grateful. In particular, I would like to thank Dr. Whiteside of the Peterborough Civic Hospital for the use of facilities to finish this paper, and Dr. Spaulding of Toronto General Hospital for reading the draft.

REFERENCES

1. **Bernstein, A.: Medicine, 19: 85-159, 1940.**
2. **Belk, W. P.: J. Lab. & Clinical Medicine, 20: 953-1042, 1935.**
3. **Dacie, J. V.: Practical Haemotology. London, Churchill. 1950.**
4. **Dougherty, T. F., Maile, J. B., Roberts, J. C., Dameskeket: Int. Acad. of Path., Monograph (Hober). 1960.**
5. **Doan, J. K., and Weisberger: Int. Med., 38: 1058, 1935.**
6. **Jenkins, W. J., Marsh, W. L., Noades, J., Tippitt, P., Sanger, R., Race, R. R.: Vox. Sang., 5: 97, 1960.**
7. **Marsh, W. L.: Brit. J. Haemat., 7: 200-9; 1961.**
8. **Tippett, P., Noades, J., Sanger, R., Race, R. R., Sausais, L., Holman, C.A., & Buttimer, R. J.: Vox. Sang., 5: 107, 1960.**
9. **Marsh, W. L., Personal communication.**
10. **Sawitsky, A., Papps, and Weiner, L. M: Ann. Med., 8: 200, 1950.**
11. **Weiner, A. S., Unger, L. J., Cohen, L., and Fildman, J.: Ann. Intern. Med., 44: 221, 1961.**
12. **Weiner, W., Battery, D. A., Cleghorn, T. E., Mason, F. G. W., and Meynell, C.: Brit. Med. J., 11: 125, 1953.**
13. **Wright, C. S., Dodd, M. C., Bournocle, B. A., Doan, C. A.: J. Lab & Clin. Med., 37: 165-181, 1951.**
14. **Smith, C. H.: Blood Diseases of Infancy and Children. The C.V. Mackey Company. 1960.**

Abstracts

Ocular Complications of Diabetes

Hausler, H. R.: Modern Med. 16: 59-61, 1961.

The author briefly summarized ocular changes in diabetes which include:

1. Extraocular muscle palsies secondary to oculomotor neuritis.

2. Swelling and degeneration of the pigment epithelium of the iris leading to proliferation of blood vessels in the iris.

3. Lenticular opacities or transient changes in the refractive power of the lens.

4. Retinopathy — the most common ocular complication manifested by microaneurisms, exudates, retinal hemorrhages and finally retinal detachement. It cannot be prevented, controlled or effectively treated.

5. Optic neuritis resulting in central scotoma.

M.T.

Spondyloarthritis in Childhood

Moes, C. A. F.: Amer. J. Roentg. 91: 578-587, 1964.

Spondyloarthritis is an inflammatory spinal disease of early childhood with typical clinical and roentgenologic findings and a rapid course. The author reports on a study of 20 patients to illustrate these characteristics.

Presenting signs include low back, hip, or abdominal pain or meningeal signs with irritability, anorexia, fever and easy fatigability. Physical examination reveals a moderately ill child preferring to lie supine with hips flexed. There is tenderness over the involved (usually lumbar) region and movements of the spine or leg accentuate the pain. Leukocytosis is slight but ESR is consistently elevated. Roentgenological signs appear 2-4 weeks after the onset of symptoms and show disk space narrowing followed by under-mineralization of adjacent vertebral margins. Eventually vertebral margins become more distinct with remineralization but disk space narrowing may persist.

Spondyloarthritis must be differentiated from tuberculosis, osteochondritis, and uncommonly, osteomyelitis. Treatment includes back supports during the acute phase and possibly antibiotic therapy.

M.T.

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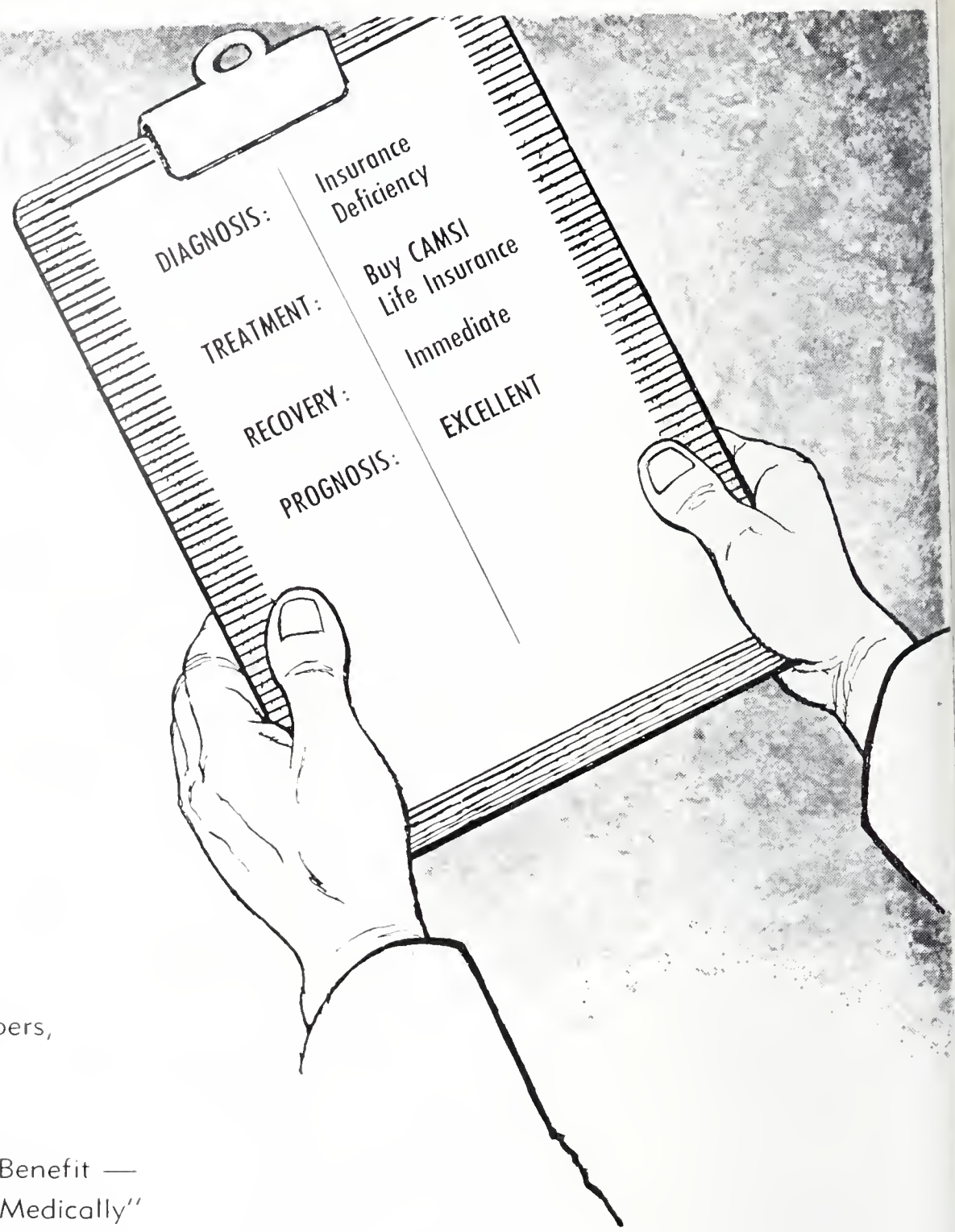

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Sutureless Prosthetic Heart Valves

INTRODUCTION

A new ball valve has been designed which can be held in place mechanically by means of multiple curved pins instead of sutures. This valve can be inserted with a special instrument in a minimum of time, can be repositioned if necessary, and does not require any cloth around its base for suturing.

DESCRIPTION

The valve consists essentially of a Silastic ball, a circular base on which the ball seats itself, and an open cage in which the ball may travel freely. The cage is formed of three or four perpendicular rods equally spaced around the upper surface of the base. The free ends of these rods curve inwards to retain the ball but they do not join each other. Consequently, the cage is "open" at the top.

The base of the valve is composed of two rings which are threaded on the outside of a central cylinder, one by a right handed thread and the other by a left handed thread. Thus, when the central cylinder alone is rotated the two rings are moved either together or apart depending on the direction of rotation.

Around the adjacent surfaces of the two rings are attached up to 24 curved pins depending on the size of the valve. The pins on the upper ring curve downwards and outwards; those on the lower ring curve upwards and outwards. When the two rings are held apart from each other the tips of the pins do not project beyond the outside of the valve base. The pins of the upper and lower rings interdigitate alternately with each other around the valve. As the centre cylinder is rotated and the rings are forced closer to each other, each ring strikes the curved part of the opposing set of pins and deflects them so that they project radially outwards beyond the circumference of the valve base. Thus the surrounding tissue is gripped firmly between the alternate upper and lower pins.

A thin silicone cuff is placed around the base of the valve to improve the seal between the valve and the surrounding tissue.

INSERTION

A special instrument is used to insert the valves in the heart. This rotates the central cylinder and at the same time prevents the outside rings from turning.

The insertion of a valve in the aortic position is accomplished through an incision in the aorta. The valve must therefore be held partly by the cage. After first removing the Silastic ball, the special tool is inserted between the rods of the cage into the central cylinder. If the existing aortic valve leaflets are badly calcified they are removed and the largest ball valve that will fit snugly into the aorticoventricular junction is inserted. A purse string suture around the opening may be used to draw the surrounding tissue more tightly around the valve base. The pins are then extended by rotating the tool, the tool is removed and the ball is replaced in the cage.

The insertion of a valve in the mitral position is done from the left atrium after removing most of the existing mitral valve cusps. In this case a slightly different tool which holds the valve from beneath the base is used. The ball can, therefore, be left in place in the cage. Again a purse string suture may be drawn tight before extending the pins.

ADVANTAGES

There are several advantages in using this type of valve fixation.

(1) Once in the correct position, the valve can be rapidly fixed in place by rotating the special tool. The speed with which the valves can be inserted lessens the period of pulmonary bypass required, lessens the need for profound hypothermia, and lessens the technical demands of coronary perfusion. Consequently, this helps reduce post-operative complications.

(2) If the initial position of the valve is not quite satisfactory, the pins can be withdrawn, the valve repositioned, and the pins re-extended.

(3) The absence of any cloth around the valve base, eliminates many possible sites of thrombus formation.

(4) The "open cage" of the valve further reduces the likelihood of thrombus formation on the cage beyond the ball.

(5) The absence of cloth also permits a somewhat larger internal opening in valves of equal external diameters as compared with other valves having a cloth ring for suturing.

SUMMARY OF RESULTS

The following results were reported in April, 1964. They include cases in which earlier models of the valves were used and, therefore, the more recent results are even more encouraging.

Fifty-two patients received these valves and of these, thirty-five were still living in April, 1964. The longest post-operative survival time was eighteen months and this patient was still living in April. None of the hospital deaths were attributed to valvular misfunction and no cases of the "stuck ball" syndrome had been observed. In no case had there been any evidence of movement or migration of the valve after insertion.

C. C. Wells 6T6

REFERENCES

1. Magovern, G. J., Kent, E. M., and Cromie, H. W. Circulation, 27: 784, 1963.
2. Magovern, G. J., and Cromie, H. W. J. Thoracic and Cardiovascular Surgery, 46: 726, 1963.
3. Magovern, G. J., Kent, E. M., Cromie, H. W., Cushing, W. B., and Scott, S. J. Thoracic and Cardiovascular Surgery, 48: —, 1964.

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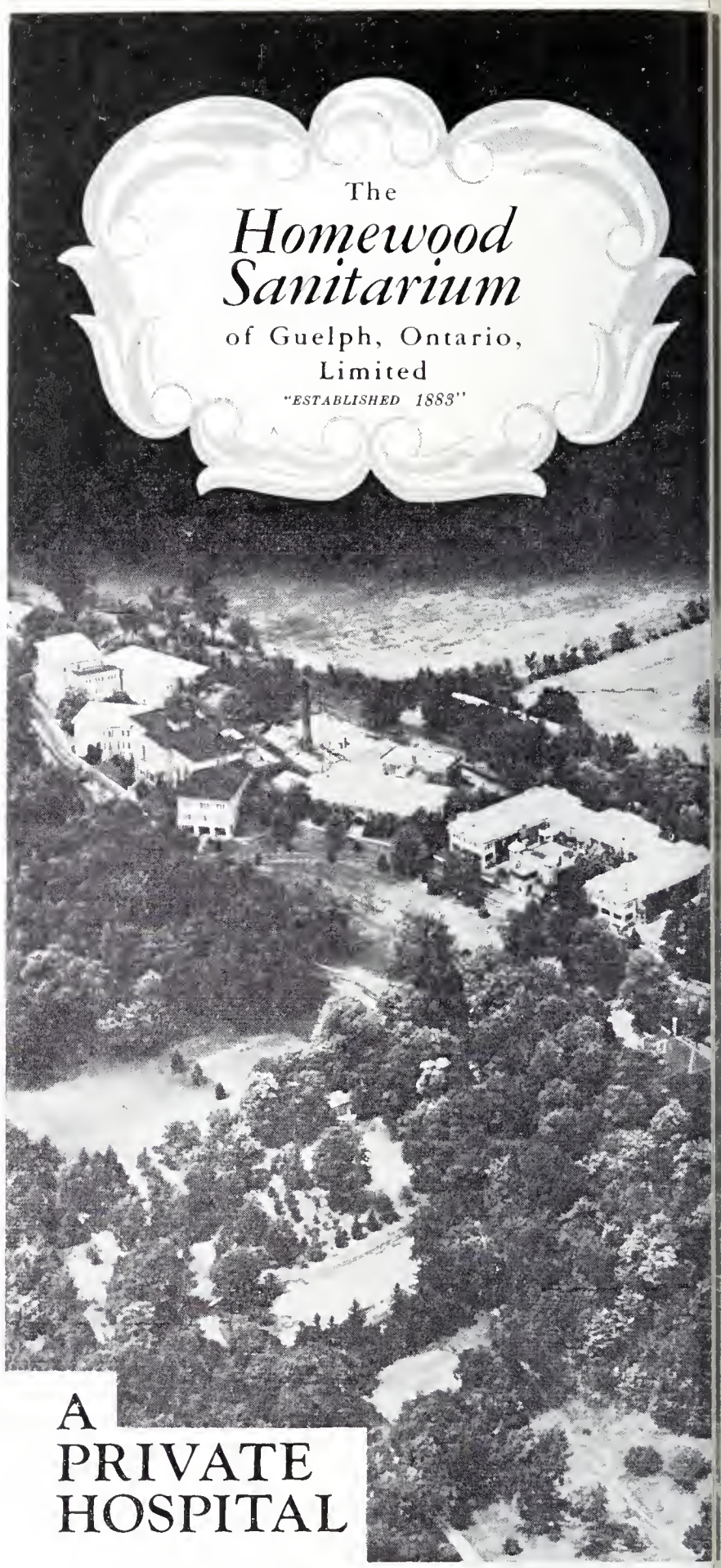

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G. S. BURTON, M.D.C.M., *Medical Superintendent.*

Serotonin in the Central Nervous System

G. R. Van Loon, B.Sc. 6T5

It has long been recognized that the effects of a drug are never produced entirely by the drug itself, but are dependent on an interaction between the drug and the organ or system affected. In the brain, with its almost infinite variability of response, we are forced to a realization of the importance of the structure or substrate upon which a drug acts, for the brain is a substrate that is infinitely more complicated than the other substrates of the body. In addition to physiology and biochemistry we are dealing with personality, or mood, or affect, all of which are merely convenient terms for that highly complex and most individualized physicochemical system, which reflects the totality of constitution plus the storehouse of past experience mingling with present awareness.

It is with this in mind that we examine the newest of neurohormones, serotonin (5- hydroxytryptamine or 5- HT).

Until recently, acetylcholine has been the sole neurohormone established for the central nervous system (CNS), but the discoveries in the brain of serotonin and noradrenaline have opened new avenues to explain the diverse effects of centrally acting drugs. A function for brain serotonin and noradrenaline is suggested from the physiologic concepts of Hess[2].

Hess pointed out that the autonomic nervous system (ANS) is not an isolated entity, since its sympathetic and parasympathetic components must synchronize centrally with the somatic nervous system in order to maintain body integrity. Thinking in terms of a coordinated process also enables us to understand the interplay between the sympathetic and parasympathetic systems. However, instead of the separation into sympathetic and parasympathetic systems which is based on morphologic criteria, terms having a functional significance may be more appropriate. Hess studied the nature of the coordination in unanesthetized cats by electrically stimulating various brain areas. From the behavioural patterns, Hess postulated that reactions of the mammalian organism to environmental changes are controlled by a subcortical system integrating autonomic, somatic and psychic functions. The subcortical system he suggested consists of two mutually antagonistic divisions — ergotropic and trophotropic. According to Hess, then, the trophotropic division integrates parasympathetic with somatomotor activities to produce behavioural patterns that conserve and restore energy; in contrast, the ergotropic division integrates sympathetic with somatomotor activities to produce patterns of behaviour which prepare the body for positive action. Both neuronal divisions are in continuous opposition, the resulting level of activity ranging from trophotropic predominance (sleep or apathy) to ergotropic predominance (excitement or mania).

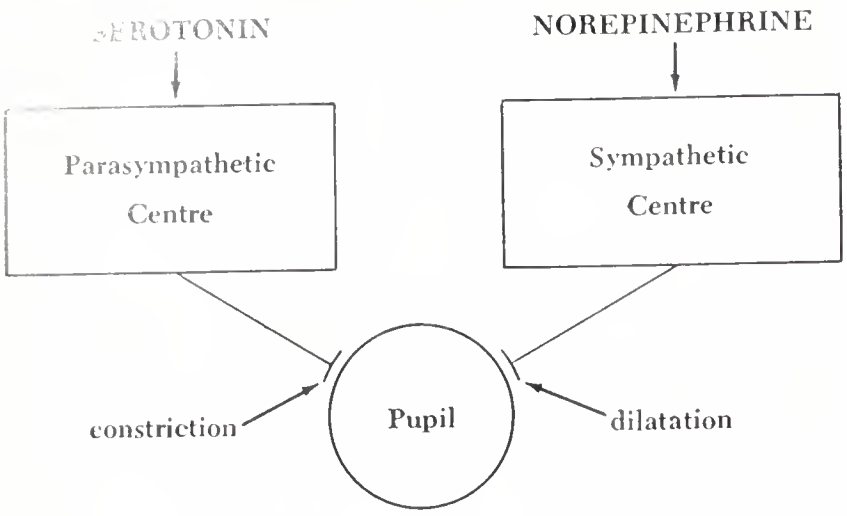
Without going into details of individual functions, it may be said that the effects elicited by stimulation of a region between the posterior and medial hypothalamus are transmitted to effector organs almost exclusively by way of the sympathetic system and that it is appropriate to speak of this as an ergotropic zone. On the other hand, the anterior and lateral hypothalamus, including the pre-optic and supra-optic areas and the septum, are responsible for the protective mechanisms and for the control of the activity of the digestive apparatus which serves as an organ of restitution. That similar effects can also be elicited occasionally by stimulation of the medial and posterior hypothalamus is understandable, since it is here that the connections are to be found which conduct the stimuli from their points of origin to the medulla oblongata and thence to the parasympathetic system. Since even the highest centers can be inhibited by application of a stimulus to the diencephalon, with the production of a condition resembling natural sleep, it can hardly be wrong to regard the diencephalon, rather than the cortex, as the true central organ of the body. Thus, it may be said that true autonomic regulation finds its highest level of direct and automatic coordination in the diencephalon.

Considerable data favour the concept that serotonin and noradrenaline (NA) may serve as neuro-hormones of these two neuronal systems, noradrenaline in the ergotropic and serotonin in the trophotropic system.

The amines, as well as the enzymes involved in their formation, have their highest concentration in the brain stem, especially in the hypothalamus where a major part of somato-autonomic integration occurs. The concept of Brodie and his co-workers, implicating serotonin and noradrenaline as chemical mediators of mutually antagonistic centers that regulate homeostatic mechanisms in the brain (Fig. 1), endeavours to explain the actions of the tranquilizing agents reserpine and chlorpromazine and the hallucinogenic agents LSD and mescaline, in terms of inter-actions with 5-HT and NA in the CNS (Fig. 2). Althought this concept is only a working hypothesis, it has already proved useful in linking a number of unrelated observations and in suggesting certain experiments that might test it.

Although it is the central actions of serotonin that are mainly being discussed here, it should be pointed

Figure 1. Mutually Antagonistic Brain Centres in the Central Autonomic Nervous System.



After Brodie B.B. and P.A. Shore, Ann. New York Acad. Sc. 66: 638, 1957.

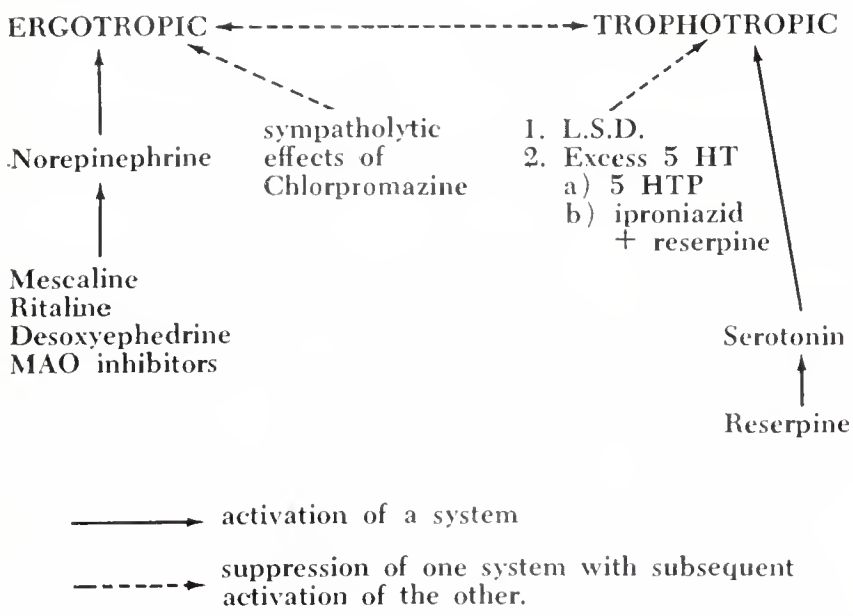
out that most of the serotonin in the body is found peripherally. Most of this is found in the mucosa of the stomach and intestine where it apparently plays great roles in gastric secretion[3] and intestinal motility.[4] Smaller amounts are found in the blood platelets which take it up as it is released from the tissues.[5] Thus, although the role of serotonin in the brain may be an important one it should not be assumed that this is its only role or even its most important one.[6]

PRESENCE IN BRAIN

The clue pointing to a role for serotonin in the functioning of the CNS came from its discovery in the brain. Serotonin was first detected in brain, using bioassay procedures, by Twarog and Page[7] and by Amin et al[8], and their findings were confirmed fluorometrically by Bogdanski et al[9].

The pathway for biosynthesis of serotonin was elucidated largely from studies on peripheral tissues and

Figure 2. Activation and Supression of Ergotropic and Trophotropic Systems.



microorganisms[10]. Tryptophan, the dietary precursor, is first oxidized to 5-hydroxytryptophan (5- HTP) and then decarboxylated to yield serotonin.

Serotonin is inactivated in the body by monoamine oxidase (MAO), the major end-product being 5-hydroxyindoleacetic acid (5-HIAA). MAO has a wide scope of action and is responsible for the inactivation of adrenaline, noradrenaline and tryptamine. The first potent inhibitor of MAO to be described was isopropylisonicotinyl hydrazine (iproniazid)[11] which was shown to potentiate the peripheral and central actions of serotonin by blocking serotonin metabolism; when administered to animals it produced a marked rise in the level of brain serotonin.[12]

In the brain, serotonin is not randomly distributed but is found localized in the brain stem areas, particularly the hypothalamus and midbrain.[13 14]

The specific localization of serotonin and the enzyme 5-HTP decarboxylase suggests that the function of this amine is very specific. It has been suggested that serotonin is stored in the brain in "bound" form and subject to release by certain agents.[15]

BINDING AND STORAGE

Almost all aspects of the physiology, pharmacology and biochemistry of serotonin or any other biogenic amine can be discussed from the point of view of their binding to tissue or tissue components. A binding mechanism has been implicated or demonstrated in the biosynthesis, storage, transport and release of amines, in their interaction with specific receptors or enzymes, in their inactivation by enzymes, and perhaps in their inactivation by binding to tissues.[16]

The same cells that contain biogenic amines almost invariably contain enzymes that inactivate the amines. Thus, it would seem that the amines must be held in a protected form in cells. In the brain most of the 5-HT was shown to be associated with granular material sedimenting with mitochondria. Probably serotonin is synthesized in part outside the granules and after synthesis becomes bound to the granules. During synthesis the molecules must shuttle between soluble cytoplasm and particulate material. Thus, tryptophane is oxidized by undefined particulate material to form 5-HTP which is then decarboxylated in the supernatant fraction to 5-HT which in turn is conveyed to the granules. During the shuttling 5-HT risks destruction by MAO in particulate material. How the amines are spared destruction and what directs the shuttling are unanswered questions.

It is possible that the final levels of serotonin in cells represents the 5-HT which perchance has escaped inactivation. Another possible explanation is that during transit between clear cytoplasm and granules the 5-HT is bound in a linkage which makes it invulnerable to enzymatic destruction. Alternatively, the biosynthetic

process may be oriented in juxtaposition with the granule or may direct the amines to the granule. The likelihood of a highly organized system in the synthesis and intracellular transport of amines is strengthened by observations suggesting that the cells may handle endogenous and exogenous amines differently.[17]

The storage of serotonin in granules appears to be connected with the storage of adenosine phosphates and protein. Put and Meduski suggest a mechanism by which the corticosteroids of the water-insoluble particulate material may control an equilibrium between bound and free serotonin, shifting the reaction to the left or to the right by bringing about the changes of the dielectric constant of the particulate material. When the cortiocosteroid moiety of the particulate material disappears (practically all tissue and plasma corticosteroid are bound by proteins[18]), as a consequence of the depletion, for example, after adrenalectomy or in Addison's disease, then impairment of the binding sites of the 5-HT obviously occurs. As a result, not only the impossibility of binding the 5-HT in a physiologically inactive form is observed (due to lack of corticosteroids), but also the decrease of the 5-HT level in the brain (due to lack of negative binding sites of the particles which is due to lack of ATP-like compounds).[19]

The association of the bulk (70%) of endogenous serotonin with the particulate fraction of homogenates of the rate brain has been reported by Giarman and Schanberg.[20] Various drugs exert different actions on the free and bound forms of 5-HT. Reserpine produces an increase in the percentage of free 5-HT while lowering total cerebral 5-HT; phenobarbital leads to an increase in both free and total 5-HT; chlorpromazine is associated with an increase in free 5-HT but does not alter total 5-HT. The results with chlorpromazine seem to fit well with the suggestion of Gey and Pletscher[21] that this drug acts to change the permeability of the storage organelles for the monoamines; that is, chlorpromazine does not alter total 5-HT, but by changing permeability of storage organelles it increases the percentage of free 5-HT. It would seem, therefore, that the subcellular distribution of 5-HT may be a more reliable correlate of the effect of a particular drug on the overall activity of the CNS than the total 5-HT level in the brain.

It should be emphasized that there is no proof as yet that 5-HT acts primarily as a transmitter. It is possible that this and other neurohumoral amines may function as neuroregulatory substances by modifying the environment in which transmitters act. Ordinarily the free form is considered to be the active form. It has been suggested, however, that the free 5-HT of the supernatant fraction is a transition form, potentially active, potentially storable, and potentially destructible. In this same view, the particulate fraction may be an estimate of the stored neurohumor (granule-bound) and the active neuro-humor (complexed with specific receptors leading to the evoked response). Thus, the neuroregulatory action of 5-HT possibly depends more upon the relative concentration-distribution among these three forms than upon any single form.[22]

RELEASE

It is thought that humoral agents are released in response to either nervous or chemical stimuli, and then carried to the receptor site where they exert their action. Administration of reserpine results in increased urinary excretion of 5-HIAA[23]. This suggests that bound serotonin has been released and metabolized; a second dose produce no increase in excretion of 5-HIAA. It was noted that the pharmacological effects of reserpine (Table 1) and the change in cerebral serotonin levels persisted long after reserpine had disappeared from brain tissue.[24,25] Shore et al[26] illustrated that one molecule of reserpine releases hundreds of serotonin molecules, thus suggesting that the release is not a simple displacement. The primary action of reserpine seems to be to impair the capacity of cells to bind serotonin[27] and, hence, its action perists until the receptors are restored.

Table 1

Actions of Reserpine

1. **sedation**
2. **generalized reduction in motor activity**
3. **↓ depth and rate of respiration**
4. **potentiation of hypnotics**
5. **parasympathetic-like effects**
   1. **↓ arterial B.P.**
   2. **↓ heart rate**
   3. **↓ body temperature**
   4. **constriction of pupils**
   5. **ptosis**
   6. **relaxation of nictitating membrane**
   7. **↑ lacrimation and salivation**
6. **↑ GI motility**

If reserpine action is mediated by serotonin, then 5-HT is normally involved in regulation of centres of wakefulness, temperature regulation, B.P. regulation, and other autonomic functions.

Administration of reserpine causes a lowering of serotonin levels in various tissues even though the amine is stored in several very different types of cell—the enterochromaffin cell of the intestine, the blood platelet, and cells of an unknown nature in the brain. Reserpine lowers the content of 5-HT and catechols in tissue, possibly by blocking the transport mechanism which pumps the amines across a membrane into the cell. Depletion of the amine content of a tissue, then

is pictured as a consequence of the uncompensated leaking of the amine from its intracellular pool.

The finding that reserpine causes a release of tissue serotonin and catecholamines, and the realization that monoamine oxidase (MAO) is involved in the metabolism of these amines, led to experiments on the effects of reserpine on animals pretreated with an MAO inhibitor, such as iproniazid. It was observed that this combination of drugs prevents the reserpine-induced lowering of amine levels, and, furthermore, causes excitation and a rise in blood pressure rather than the central depression and hypotension seen after administration of reserpine alone. The ability of MAO inhibitors to abolish or even reverse the action of reserpine when the inhibitors are given before, but not after, reserpine was assumed to be due to inability of the brain to metabolize the amines which are released by reserpine, the amine then being in a free or active state.

In summary, then, serotonin is in equilibrium between its free and bound forms. Reserpine or MAO inhibitor (iproniazid), acting at their respective different sites, upset this balance in such a way that a persistent low level of free 5-HT exists. It is this persistent low level of free 5-HT which produces the trophotropic actions.

Thus, Shore et al[28] suggested that the effect of reserpine is accounted for by the continued presence of *free serotonin*. Evidence for this is as follows[29]:

1. Sedative and potentiation effects of reserpine are mimicked by parenteral injection of 5-HT; mice recovered while the brain 5-HT level remained high. The increase presumably represents bound and therefore pharmacologically inactive 5-HT;

2. Change in level of bound 5-HT in brain did not parallel pharmacological effects of reserpine;

3. 5-HTP added to rat uterus resulted in an increased level of 5-HT but no contraction, whereas a minute amount of 5-HT added to the solution in which the uterus was suspended produced contraction;

4. Reserpine followed by serotonin caused a profound decrease in blood pressure. This is interpreted as follows: a small amount of free 5-HT passed into brain where it could not be bound readily, adding to the effect of free 5-HT already present as a result of reserpine action;

5. Iproniazid followed by reserpine produced excitation and sympathetic effects similar to the effects of LSD or high doses of 5-HTP. Such effects are associated with high concentration of free 5-HT in brain.[30]

The release of serotonin by reserpine is not duplicated by chlorpromazine, LSD, or phenobarbital. Pletscher and Gey[31 32], in pointing out that chlorpromazine inhibits the action of neither MAO nor decarboxylase enzyme nor the penetration of MAO inhibitors into brain, concluded that chlorpromazine may act by decreasing the permeability of granules in which the amines are stored.

A recent report of Gursey and Olson[33] presented evidence that alcohol produces a depression of serotonin levels in the brain stem of rabbits. Further reports by Häggendal and Lindqvist[34] and Pscheidt, Issekutz, and Himwich[35] failed to confirm these results. Similarity between alcohol and reserpine in depleting serotonin would suggest a mechanism for the hypnotic and sedative effects of alcohol. Further work in this area was obviously indicated and it was on this basis that the author, with the guidance of Dr. H. Kalant and Dr. R. Hawkins, attempted to show the effect of acute alcohol intoxication on serotonin levels in brain stem of rabbits. Our results did not show a significant depression of brain stem serotonin levels by oral ethanol in rabbits. The relation between alcohol, serotonin and central nervous system function remains to be revealed.

PROPOSED MECHANISM OF ACTION

Woolley[47] has presented evidence which suggests that the basic mechanism of action of serotonin seems to be to arrange for the entry of calcium ions into cells. Serotonin may combine with a specific lipid of the cell membrane, producing a serotonin-receptor complex which in turn forms a salt or chelate with calcium ions, thus rendering the calcium fat-soluble. A receptor enzyme would then be required to act upon this calcium-serotonin-receptor substrate, destroying part of the complex, releasing the calcium ion and liberating it into the aqueous phase within the cell. An additional enzyme would be required to regenerate the receptor substance, so that the next cycle of transport could take place. A spatially alternated network of these two enzymes situated back to back on the cell membrane would present a cyclic process of calcium transport into and out of the cell. Such a mechanism would momentarily give an intracellular concentration of calcium great enough to produce depolorization. Experimental data substantiating this hypothesis of the biochemical mechanism of action of serotonin is incomplete.

THE ROLE OF SEROTONIN AS A CHEMICAL MEDIATOR IN THE BRAIN

Circumstantial evidence, but little direct proof, exists for the view that serotonin acts as a transmitter in mammals. Brodie and Shore[36] have proposed the concept that 5-HT is the chemical transmitter of the central parasympathetic system, with noradrenaline the central sympathetic transmitter. The following reasons are given[37]:

1. Serotonin is present in the brain;

2. Serotonin is present in highest concentration in the brain stem where the major part of autonomic integration occurs;

3. MAO, which destroys 5-HT, is especially concentrated in the hypothalamus;

4. 5-HTP decarboxylase, which is able to synthesize 5-HT, varies in parallel with the cerebral 5-HT content;

5. LSD antagonizes the central effects of administered serotonin and evokes marked central sympathetic activity;

6. When large doses of 5-HT are given to mice intraperitoneally, a small quantity penetrates the brain and the mice become sedated; hence, the addition of the transmitter to brain has a demonstrable central effect;

7. Inhibiting the inactivating enzyme, MAO, by iproniazid and then giving reserpine to release cerebral 5-HT does not elicit the usual effects of reserpine. On the contrary, the response is typical of LSD, including violent excitation, mydriasis, exophthalmos, piloerection, and other signs of sympathetic activity. Also, administration of a large amount of 5-HTP leads to high levels of 5-HT in brain, resulting in a behaviour similar to that elicited by LSD. It can be seen that in both of these situations an excess of free serotonin results .The antiserotonin action of serotonin in excess may be analogous to the well known inhibition of an enzyme. by an excess of its substrate. With the hormones, one molecule ordinarily combines with one molecule of receptor at its active site. If a large excess of the hormone is present, several molecules of it may attach to the single active site of the receptor and result in an abnormal reaction. LSD does not prevent the release of serotonin, but probably acts by blocking the action of liberated 5-HT, possibly by competitive inhibition.

Chemical mediators such as acetylcholine are considered to work through the membrane depolarization of nerve cells. Thus, in the brain stem, nerve impulses along presynaptic fibres might release serotonin in minute amounts at synaptic junctions. This release of free serotonin might act, in the way previously suggested, as a chemical mediator of responsive brain centers that in turn send impulses along postsynaptic fibres.

SEROTONIN AND MENTAL ILLNESS

There is no proof of serotonin control of normal mental processes, only a body of evidence which indicates that tampering with the functioning of this hormone results in behavioural changes in man and animals.[30] Evidence for participation of 5-HT in mental disease is as follows[37]:

1. Woolley and Shaw[39] suggested the association on the basis that many antimetabolites of 5-HT produce mental disturbance;

2. LSD is halucinogenic and Gaddum[40] showed that LSD was a powerful antagonist of 5-HT; this led to the postulation that in order to maintain sanity, 5-HT must be available to the brain;

3. Brodie[41] pointed out that reserpine liberates cerebral 5-HT and this release is associated with the tranquilizing effects of 5-HT;

4. Marrazzi and Hart's[42] work showed that 5-HT more effectively than any other agent depressed synaptic transmission within brain, suggesting that it is this interference with communication that causes mental disturbance;

5. Elevation in brain serotonin levels results in behavioral changes such as muscle tremors, salivation, tachycardia, pupillary dilation, etc. This elevation may be achieved either by the administration of 5-HTP which crosses the blood-brain barrier and is converted to 5-HT by decarboxylase[43] or iproniazid which protects 5-HT from destruction[44];

6. Other indoleakylamine-like substances; eg., adrenochrome; can produce psychosis.

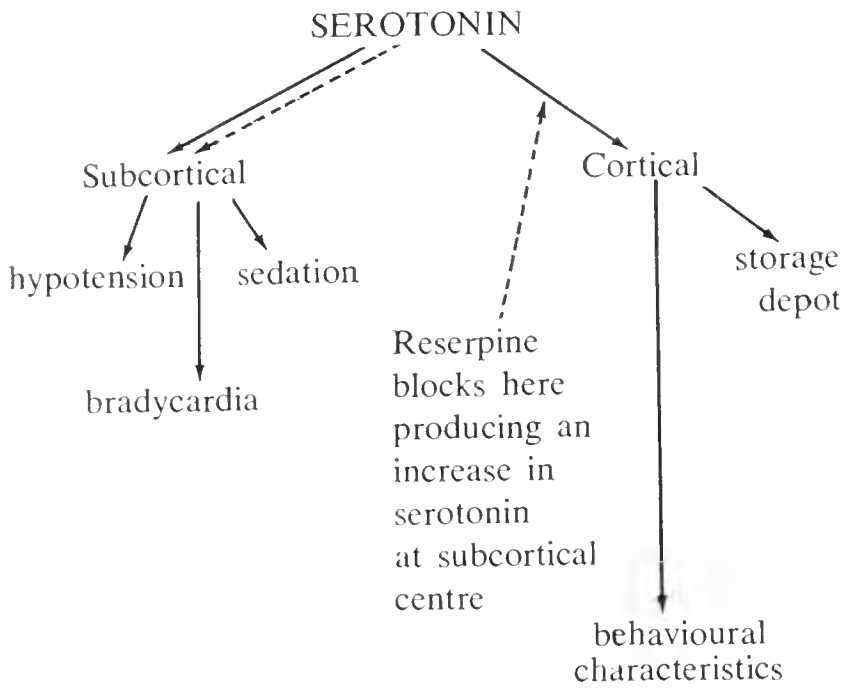
According to Wooley's[47] serotonin hypothesis, schizophrenia is the result of an abnormality in the metabolism of serotonin in the brain. The mental abnormality is regarded as being the result of changes, brought about by abnormal serotonin levels, in the functions of cells in the central nervous system, particularly the oligodendroglial cells. It is felt that schizophrenia starts, the tendency possibly being inborn, with a failure to form enough serotonin in the brain, and this is seen as the shyness and depression which are usually the forerunners of the disease. With sharply increased emotional strain, the control mechanism which governs the level of serotonin in the brain begins to fail. The production of serotonin may increase sharply (or its rate of destruction may decrease) and this probably coincides with the agitation and hallucinations. Reverberations in the form of changes in the concentrations of other neurohormones may interfere and accentuate the disease. A similar mechanism might be proposed for the manic and depressed phases of manic depressive psychosis, and according to this view, there would be no fundamental difference between schizophrenia and manic depressive psychosis. Both would be pictured as errors in the metabolism of serotonin in the brain. The differences of thought and behaviour, which have been used in the past to distinguish the two diseases, would be regarded as arising from secondary modification of the basic fault.

Also, it might be noted that there is indirect evidence that elevated serotonin may play some role in grand mal epilepsy.

If the role of serotonin (or norepinephrine) in brain function is that of a chemical mediator, especially of subcortical centres, it seems unlikely that the many kinds of mental illnesses could possibly be explained by the single premise of faulty nerve transmission; this seems too easy a solution of the problem. The chemistry of the brain that could account for the specialized functions capable of memory, evaluation, and selection

is undoubtedly extraordinarily complicated. While the abundance of research with the tranquilizing and hallucinogenic agents and with serototnin may not lead to a sound understanding of schizophrenia, it may well result in a better comprehension of the integration of the subcortical centers in the brain that regulate the autonomic homeostatic mechanisms.[45]

Figure 3. Functional Centres in CNS Utilizing Serotonin



By study of the chemistry of the brain, we seek to define the mechanisms that result in the transmission and integration of nerve impulses and, concurrently, to define their association with thought, no matter what association that may be. The danger quite obviously lies in the nature of the problem of thought itself. The mind-body dualism has received no solution, but at least it is under investigation. Whether mind is only another property of protoplasm, or whether it is an intangible stuff not lending itself to such material approaches as those of science, will be answered eventualy; the point is that the search has started. Whether the brain *secretes* thought or is only the substrate that provides the means of expressing it is another of the persistent problems. But, the least common denominator is that the nervous system requires energy which is chemical, and this is subject to scientific analysis. If we are content to do this competently, a great contribution will have been made.[46]

REFERENCES

1. Kety, S. S.: N.Y. Acad. Sc., 66: 836, 1957.
2. Hess, W. R.: Diencephalon, Autonomic and Extrapyramidal Functions. New York, Grune E. S. and Stratton. 1954, pp 59-63.
3. Haverback, B. J. and D. F. Bogdanski: Proc. Soc. Exp. Biol., 95: 392, 1957.
4. Bulbring, E. and R.C.H. Lin: J. Physiol., 138: 12P, 1957.
5. Weissbach, H., Bogdanski, D. F. and S. Udenfriend Arch. Biochem. Biophys., 73: 492, 1958.
6. Udenfriend, S.: Serotonin Metabolism and the Centra Nervous System IN Biochemistry of the Central Nervous System, ed. F. Brucke. New York, Pergamon Press (1959).
7. Twarog, B. M. and I. H. Page: Amer. J. Physiol., 175 157, 1953.
8. Amin, A. H.; Crawford, T. B. B. and J. H. Gaddum J. Physiol, 126: 596, 1954.
9. Bogdanski, D. F., Plescher, A., Brodie, B. B. and S Udenfriend: J. Pharmacol. Exp. Therap., 117: 82, 1956
10. Mitoma, C., Weissbach, H. and S. Udenfriend: Arch Biochem. Biophys., 63: 122, 1956.
11. Zeller, E. A., Barsky, J. and E. R. Berman: J. Biol Chem., 214: 267, 1955.
12. Udenfriend, S. and D. F. Bogdanski: J. Pharmacol. 120: 255, 1955.
13. Bogdanski, D. F.; Weissbach, H. and S. Udenfriend J. Neurochem., 1: 272, 1957.
14. Gaddum, J. H. and N. J. Giarman: Brit. J. Pharmacol. 11: 88, 1956.
15. Shore, P. A., Silver, S. L. and B. B. Brodie: Science 122: 284, 1955.
16. Green, J. P.: Advances in Pharmacol., 1: 349, 1962
17. Green, J. P.: Ibid.
18. Daughaday, W. H.: J. Clin. Invest., 37: 519, 1958.
19. Put, T. R. and J. W. Meduski: Acta Physiol. Pharmacol., Neerl., 11: 240, 1962.
20. Giarman, N. J. and S. Schanberg: Biochem. Pharmacol. 1: 301, 1958.
21. Gey, K. F. and A. Pletscher: J. Pharmacol., 133: 18, 1961.
22. Schanberg, S. and N. J. Giarman: Biochem. Pharmacol., 11: 187, 1962.
23. Shore, P. A., Silver, S. L. and B. B. Brodie: Science, 122: 284, 1955.
24. Brodie, B. B., Pletscher, A. and P. A. Shore: J. Pharmacol. Exp. Therap., 116: 9, 1956.
25. Hess, S. M., Shore, P. A. and B. B. Brodie: J. Pharmacol. Exp. Therap., 118: 84, 1956.
26. Shore, P. A., Carlsson, A. and B. B. Brodie: Fed. Proc., 15: 483, 1956.
27. Brodie, B. B.. Tomich, E. G., Kuntzman, R. and P. A. Shore: J. Pharmacol. Exp. Therap., 119: 461, 1957.
28. Shore, P. A.; Pletscher, A.; Tomich, E. G.; Carlsson, A.; Kuntzman, R. and B. B. Brodie: Ann. N.Y. Acad. Sc., 66: 609, 1957.
29. Page, I. H.: Physiol. Revs., 38: 277, 1958.
30. Shore, P. A. and B. B. Brodie: Proc. Soc. Exp. Biol. Med, 94: 433, 1957.
31. Gey, K. F. and A. Pletscher: J. Pharmacol., 133: 18, 1961.
32. Pletscher, A. and K. F. Gey: Proc. First Int. Pharmacol. Meet., Stockholm, 1961. London, Pergamon Press (in press), 1962.

33. Gursey, D. and R. E. Olson: Proc. Soc. Exp. Biol., N.Y., 104: 280, 1960.

34. Haggendal, J. and M. Lindqvist: Acta Pharmacol. et Toxicol., 18: 278, 1961.

35. Pscheidt, G. R. Issekutz, B., Jr. and H. E. Himwich: Quart. Journ. Stud. Alcohol., 22: 550, 1961.

36. Brodie, B. B. and P. A. Shore: Ann. N.Y. Acad. Sc., 66: 631, 1957.

37. Page, I. H.: Physiol. Revs., 38: 277, 1958.

38. Woolley, D. W.: Hormones, Brain Function and Behaviour, ed. H. Hoagland, New York: Academic Press (1957), p. 127.

39. Woolley, D. W. and E. Shaw: Science, 119: 587, 1954.

40. Gaddum, J. H.: J. Physiol., 121: 15, 1953.

41. Shore, P. A., Silver, S. L. and B. B. Brodie: Science, 122: 284, 1955.

42. Marrazzi, A. S. and E. R. Hart: Science, 121: 365, 1955.

43. Udenfriend, S., Weissback, H. and D. F. Bogdanski: J. Biol. Chem., 224: 803, 1957.

44. Woolley, D. W., Van Winkle, E. and E. Shaw: Proc. Nat. Acad. Sc., 43: 128, 1957.

45. Brodie, B. B. and P. A. Shore: Ann. N.Y. Acad. Sc., 66: 631, 1957.

46. Page, I. H.: Science, 125: 721, 1957.

47. Woolley, D. W.: The Biochemical Basis of Psychoses. New York, John Wiley & Sons, Inc. 1962.

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On Inspection - Answers

1. a) The first photograph shows the characteristic vertebral changes which occur in *Ankylosing Spondylitis* (Rheumatoid Spondylitis, Marie-Strumpell's Disease, Von Bechterew's Disease, and others).

b) Erosive *sacroiliac joint changes* are the first bony changes to occur in ankylosing spondylitis. They are present in all patients with this disease. This change is detected by the blurring of the normally distinct sacroiliac joint margins. These erosive changes are usually bilateral, but may appear more pronounced unilaterally.

The *vertebral changes* in ankylosing spondylitis are of two types: erosion of joint surfaces and calcification of ligaments. The joints involved are the posterior intervertebral facets, and the costovertebral and costotransverse articulations. Ligamentous calcification may affect the fibrous capsule of the facets, the outer layer of the annulus fibrosus, the anterior and posterior ligaments, the interspinous ligaments, the ligamentum flavum and even ligaments at the costovertebral joint. Calcification of the annulus fibrosus is usually first detected between the last two thoracic and first lumbar vertebrae. Another early change is "squaring" of the vertebrae.

The end result of calcification of the annulus fibrosus is the *bamboo spine* seen in Figure 1. Calcification of the anterior longitudinal ligament gives a streaky appearance to the bodies, interspinous ligament calcification causes a single dense central line, and calcification of posterior joint capsules gives the double vertical line or *train-track*. Other joint changes in ankylosing spondylitis occur in 50-60% of patients, and may be found in the feet, hips, pubic symphysis, sternum, and, less commonly, in the shoulder and hand. A lesions resembling superficial osteitis with secondary calcium deposition may be found at the ischium and inferior surface of the os calcis.

c) The most common presenting symptom of this disease is characteristically early morning low back pain (75%). There may be sciatic radiaion, without paraesthesia. The onset may be more acute and resemble an acute disc protrusion. Less often the disease may manifest itself in the chest, neck or shoulder girdle. In 10% of cases peripheral joint pain, often in the lower limb, may be the presenting symptom. A few cases have only constitutional symptoms.

The complications of ankylosing spondylitis are:

1. Iritis (in 20-40% of cases);
2. Aortitis and myocarditis (1-3%);
3. Atlanto-axial subluxation;
4. Amyloid disease (5% of autopsy cases).

Understandably, these complications occur in the more severe forms of the disease, notably when there is perpiheral joint involvement.

Important conditions to rule out in ankylosing spondylitis are Reiter's Disease, psoriatic arthritis, arthritis of ulcerative colitis and rheumatic fever.

2. a) Figure 2 illusrtates the erythema at the base of the nails which occurs in *dermatomyositis*. There may also be fingertip erythema and erythematous patches on the dorsa of the fingers. Raynaud's phenomenon may occur. Similar nail bed signs may also occur in systemic lupus erythematosus, psoriatic arthritis, rheumatoid arthritis, and Reiter's syndrome. Certain allergies, early clubbing, minor cuticular trauma, and fungal infection must be ruled out.

b) Cutaneous features of dermatomyositis occur in 65% of cases. Typically there may be oedema and dusky erythema of the face, periorbital region, neck, shoulders, upper arms and upper thorax. A red suffusion of the upper eyelid is characteristic. Other skin manifestations may be seen, ranging from transitory erythematous and bullous or haemorrhagic lesions to exfoliative dermatitis.

Glossy or atrophic red patches may be seen in the mucous membranes of mouth and pharynx (cf., mucocutaneous syndromes, Reiter's syndrome, Stevens-Johnson syndrome, and Behcet's syndrome). Less common cutaneous features include dusky, ulcerative, erythematous patches over bony points, thickening and contraction of skin of fingers and face (cf., scleroderma), telangiectasia and pigmentation. Rheumatoid nodules do not occur.

3. a) Figure 3 is an example of a *rheumatoid nodule*.

b) Essentially, the histological changes in the autoimmune disease are mucoid oedema, fibrinoid deposition, inflammation and sclerosis. The nodule of rheumatoid disease consists of a central area of *fibrinoid* surrounded by a palisade of primitive *fibroblasts*, and more peripherally by *plasma cells* and *lymphocytes*. The finding that there may be changes of arteritis in this most peripheral part of the nodule has prompted some investigators to suggest that it is an arteritis which forms the focus for fibrinoid change and nodule formation. Fibrosis and calcification of a nodule may occur in later stages of nodule formation.

In contrast to the rheumatoid nodule, a rheumatic nodule has at its centre diffuse fibrinoid and exudation surrounded by loosely organized fibroblasts, and a mild degree of inflammation. No end-arteritic changes occur.

c) Complications of rheumatoid disease.

A. Joint complications

B. Systemic complications

1. Constitutional
   a) Emaciation, anaemia and increased incidence of infection

2. Skin
   a) Shiny, atrophic, easily bruised
   b) Bronzing of face and limbs, patches of vitiligo
   c) Nodules
   d) Chronic ulcers on lower third of leg in severe cases

3. Eye
   a) Scleromalacia perforans
   b) Iridocyclitis
   c) Kerato-conjuctivits sicca (14.3%)

4. Respiratory System
   a) Hoarseness and laryngeal stridor, from involvement of crico-arytenoid cartilage
   b) Pleurisy (found in 25% of autopsy cases)
   c) Interstitial pneumonitis and nodular lung lesions

5. Cardiovascular System
   a) Vasomotor imbalance (cold and clammy hands, paraesthesia, etc.)
   b) Arterial vasculitis of 3 types:
      (i) arteries to muscles and nerves
      (ii) widespread of larger vessels
      (iii) digital arteritis
   c) Pulmonary hypertension on the basis of vascular spasm (cf., idiopathic pulmonary hypertension)
   d) Symptomatic and asymptomatic forms of carditis may occur
   e) Aortic valve ring changes

6. Bone
   a) Spontaneous fractures
   b) Carpal tunnel syndrome
   c) Cervical spine involvement
   d) Atlanto-axial dislocation

7. Muscles
   a) Primary nonspecific muscular atrophy (possibly due to arteritis)

8. Tendons
   a) Nodules, trigger finger, extensor and flexor tendon rupture, Dupuytren's contracture.

9. Gastro-intestinal System
   a) Vomiting, constipation, anorexia

10. Genito-urinary System
    a) Asymptomatic form of glomerulonephritis
    b) Amyloid disease

11. Neurological
    a) Twitching, vertigo, tremor
    b) Neuropathy (in severe disease)
    c) Asymptomatic arteritis of brain

4. a) All 3 photographs are examples of *dermatitis medicamentosa*. Figures 4 and 5 are examples of Penicillin drug allergy, Figure 6 demonstrates a Sparine ® allergic eruption.

There is a tendency to ascribe any atypical drug reaction to allergy. However, we must not confuse allergic response with drug toxic effects (e.g., gold), normal pharmacological effects manifest at a higher dose (e.g., ergot), intolerance (e.g., quinine) or idiosyncrasy.

The allergic response which the patient gets will differ from the normal pharmacodynamic effect of the drug, should disappear at a variable rate with withdrawal of the drug, and return when the individual is challenged by the antigen again. This latter fact is really the only test of drug allergy. Aside from a few specific examples of drugs which produce characteristic skin lesions (e.g., bromides, iodides, phenolphthalein, barbiturates) *every known type of skin eruption may be induced by drugs*. This eruption may be accompanied by generalized systemic signs; e.g., fever, lymphadenopathy, bone marrow depression, etc.

Penicillin sensitivity is the most common drug allergy which occurs. Some investigators have estimated that 10% of the population will have some allergic response to this drug.

b) Skin Testing

Because most drugs have a molecular weight of less than 5000, they are poor antigens in themselves. Evidence suggests that to be a complete antigen it is necessary for either the *drug* or one of its *breakdown products* to act as a hapten and combine with body protein. Recently interest has been stimulated in a *penicillin derivative* (penicilloyl) coupled to a lysine homopolymer. This compound, *penicilloyl-polylysine* (PPL), can elicit a wheal and flare skin response in a high percentage of penicillin allergic individuals. The use of PPL is far superior to haemagglutination techniques, and pure drug intradermal injection for detecting penicillin sensitivity. Patients who give any history of penicillin allergy show a 35% positive skin reaction to PPL, and those with strongly positive skin tests have a 9 times increased risk (7.7%) of a systemic reaction following penicillin treatment. In patients who have recently had an allergic penicillin reaction, PPL will give a positive skin test in 90%. Patients with no history of allergic response to penicillin therapy have a positive test in 6.8% of cases. (It has been suggested that dif-

ferent forms of penicillin may produce different intensities of allergy.) Three percent of patients who have received penicillin give a positive skin test with PPL. The results obtained with PPL are 90% reproducible in the same patients. Despite early expectations that PPL is nonantigenic in itself, there have been 5 cases reported of skin urticaria and rashes after the injection of this material. The danger of anaphylactic response therefore exists.

A history of atopy and particularly rheumatic fever appears to positively influence skin reactivity. It has been reported that a 30% incidence of atopy exist in penicillin sensitive patients, as versus 15% in non allergic. Negroes appear to have a higher incidence o positive reactions than Caucasians. Children have th lowest incidence of positive skin reactions. The influenc of the time elapsed, the dose and type of pencillin use has not been explored.

ACKNOWLEDGEMENT

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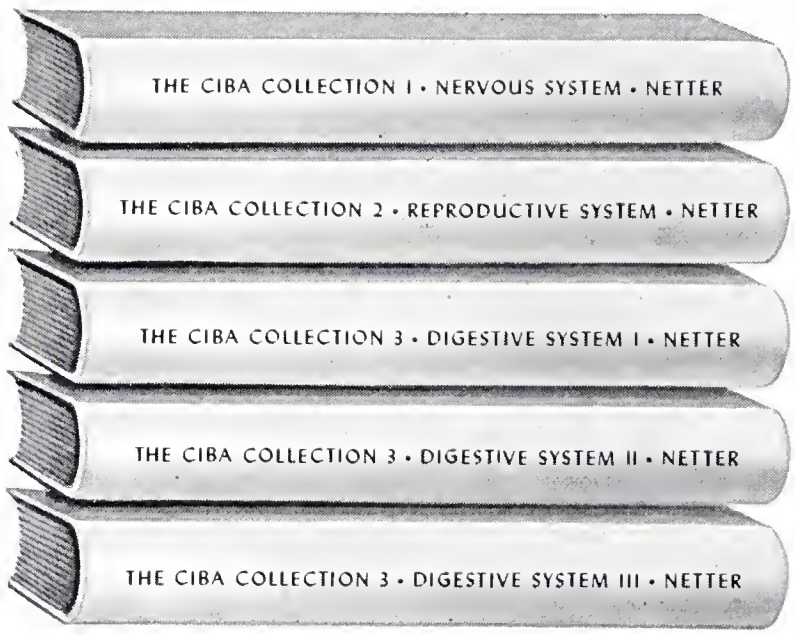
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